# VAGAL CARDIAC ACCELERATION

**occurrence**

**nature**

**significance**

A. Roossien

RIJKSUNIVERSITEIT GRONINGEN

# VAGAL CARDIAC ACCELERATION

## occurrence, nature and significance

PROEFSCHRIFT

ter verkrijging van het doctoraat in de
Geneeskunde
aan de Rijksuniversiteit Groningen
op gezag van de
Rector Magnificus Dr. F. van der Woude
in het openbaar te verdedigen op
maandag 12 december 1994
des namiddags te 4.00 uur

door

ALBERT ROOSSIEN

geboren op 24 oktober 1959
te Hoogezand-Sappemeer

**Stellingen**

behorende bij het proefschrift

**VAGAL CARDIAC ACCELERATION**
**occurrence, nature and significance**

1. Het afnemen van de "intrinsieke hartfrequentie" bij veroudering heeft een extrinsieke oorzaak.

2. Het feit dat het vagale cardio-acceleratoire systeem is opgenomen in een regelkring maakt het wachten op het verdwijnen van de vagale cardio-acceleratie[1,2] na toediening van een muscarine receptor antagonist tot een onzinnige en tijdsverslindende bezigheid.
([1] Robinson JL, Am J Physiol 1986; 251:H253-H260)
([2] Evans Y et al. Am J Physiol 1990; 258:H1132-H1137)

3. De grootte van de vagale cardio-acceleratie van een individu hangt samen met de mate waarin deze het hoofd boven het maaiveld uitsteekt.

4. Het verwijderen van gegevens uitsluitend omdat ze te veel afwijken van een gemiddelde waarde[1,2,3], is wetenschappelijk niet verantwoord.
([1] Rigel DF et al. Am J Physiol 1984; 246:H168-H173)
([2] Hill MRS et al. Ann Int Conf IEEE Eng Med Biol Soc 1990; 12:757-758)
([3] Jose AD and Collison F, Cardiovasc Res 1970; 4:160-167)

5. Bij lokale toediening van farmaca voor de bestudering van lokale effecten behoort de hoeveelheid toe te dienen stof niet te worden gerelateerd aan het lichaamsgewicht.

6. De veelheid aan perifere neurotransmitters maakt volledige blokkade van de invloed van het autonome zenuwstelsel op het hart langs farmacologische weg ondoenlijk.

7. In systeemfysiologisch onderzoek is het gebruik van anesthesie af te raden.

8. De hond verdient een stelling.

9. Het regelen van verkeerslichten door buschauffeurs bevordert het negeren van rood licht door de overige weggebruikers.

10. Het aantal lijnrechters bij voetbal moet worden vertienvoudigd.

11. Om de afstand tussen burger en politiek te verkleinen zouden beleidsnota's in stripvorm moeten worden uitgebracht.

12. Het bestaan van invloedrijke ideële organisaties loopt gevaar wanneer het publiek de verenigbaarheid van idealisme en professionele bedrijfsvoering niet inziet.

Bert Roossien

Groningen 12 december 1994

promotores: Prof. Dr. W.G. Zijlstra
Prof. Dr. J. Zaagsma

co-promotor: Dr. J.R. Brunsting

*Pour bien comprendre une science*
*il faut en connaître l'histoire*

(Auguste Comte)

promotiecommissie: Prof. Dr. B. Bohus
Prof. Dr. J.R.G. Kuipers
Prof. Dr. K.I. Lie

The studies described in this thesis have been performed at the department of Medical Physiology, University of Groningen, The Netherlands.

The research was supported by The Netherlands Organisation For Scientific Research (NWO-Medische Wetenschappen, Grant no. 900-516-106).

Financial support by the Netherlands Heart Foundation for the publication of this thesis is gratefully acknowledged.

Printing: Krips Repro, Meppel

Voor Petra en Nick

# CONTENTS

# 1

## GENERAL INTRODUCTION

The innervation of the heart is a subject which has engrossed the attention of physiologists ever since the days of Galenus (131-200). Yet, notwithstanding the great number of observers who have advanced our knowledge, there are still important points which are enveloped in obscurity. One of these points is the vagally mediated accelerator influence on heart rate. Classically, the autonomic nervous control of the heart rate is exerted through the sympathetic accelerator and the parasympathetic decelerator system. Sympathetic nerve stimulation increases the heart rate by releasing norepinephrine from the postganglionic nerve terminals, which activates post-junctional $\beta_1$- (as well as $\beta_2$-) adrenergic receptors on sinus node cells and stimulates adenylyl cyclase, thereby increasing the rate of diastolic depolarization. Parasympathetic nerve stimulation decreases the heart rate by releasing acetylcholine from postganglionic nerve terminals, which activates post-junctional muscarinic $M_2$-receptors on sinus node cells. As a consequence, adenylyl cyclase activity is decreased and certain $K^+$ channels are activated, thereby inducing hyperpolarization of the cell membrane.

The classical concept of the dual innervation with opposite functional effects is incomplete, since a positive chronotropic influence of the vagus nerve has been established (Fig. 1). This influence is not mediated by norepinephrine or acetylcholine, as demonstrated by the fact that the acceleration is observed after $\beta$-adrenergic and muscarinic receptors are blocked. Although this vagal cardiac accelerator system has been the subject of research for more than a century, its existence is still not commonly acknowledged, and even rejected, mainly because the underlying mechanism and the functional relevance of the system have not been clearly understood.

In this thesis experiments are described focused on expanding the knowledge about the vagal cardiac accelerator system. In the subsequent sections of chapter 1 the current knowledge about the vagal cardiac accelerator system is outlined. In the following chapters the results of the different studies are reported and, finally, all results are reviewed in a general discussion.

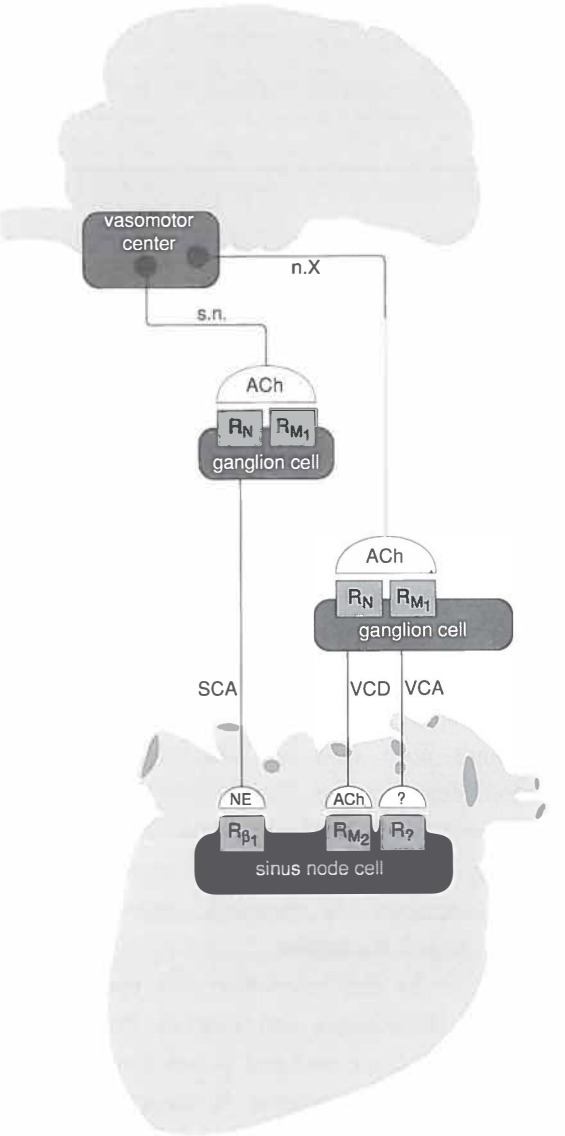



Fig. 1 Schematic representation of the autonomic nervous control of the heart rate. SCA, VCD and VCA signify the sympathetic cardiac accelerator, the vagal cardiac decelerator, and the vagal cardiac accelerator system, respectively. The sympathetic and vagal outflow from the vasomotor center of the medulla oblongata is indicated by s.n. and n.X, respectively. ACh and NE represent the classical neurotransmitters acetylcholine and norepinephrine. $R_N$ and $R_{M1}$ are, in respective, the nicotinic and $M_1$-muscarinic receptors which are localized on the membranes of sympathetic and vagal ganglion cells. $R_{\beta 1}$ and $R_{M2}$ are, in respective, $\beta_1$-adrenergic and $M_2$-muscarinic receptors which are localized on the membranes of the sinus node cells. ? and $R_?$ represent the unknown terminal transmitter and terminal receptor in the VCA system.

## 1.1. Stimulation-induced vagal cardiac acceleration

### 1.1.1. The chronotropic function of the vagus

In studying the influence of the vagus nerve, Ernst and Eduard Weber demonstrated in 1845 that electric stimulation of the peripheral end of the cut vagus nerve decreases the heart rate and may even bring the heart temporarily into a state of complete rest, and Fowelin showed in 1851 that bilateral vagotomy results in an increase in heart rate. These investigators concluded that the function of the vagus nerve is to inhibit the activity of the heart (36, 42, 44). On the other hand, Schiff (43, 44) regarded the vagus as a motor nerve of the heart, because in his experiments vagal nerve stimulation only induced an increase in heart rate. According to Schiff (44) the discrepancy in results can be explained by the use of different intensities of stimulation. An increase in heart rate is obtained when the vagus nerve is stimulated gently, while a decrease in heart rate is obtained when the vagus nerve is stimulated with great power. As a consequence, the decrease in heart rate was looked upon as a loss of the accelerator influence due to exhaustion of the vagus nerve during the too powerful stimulation.

Several scientists, such as Valsalva, Moleschott and Lister, supported the opinion of Schiff (44), while the opinion of the Webers was favored by Volkmann, Pflüger and Von Bezold (42). For the purpose of ascertaining which theory is correct, Rutherford (42) performed experiments in which he examined the chronotropic response to vagal nerve stimulation in the frog, the rabbit and in the dog. Although he carefully varied the intensity of stimulation, he only obtained a decrease in heart rate and, therefore, concluded:

*"The foregoing experiments lead me to regard the inferior cardiac branch of the vagus as an inhibitory nerve of the heart, and also to think that it cannot, in any sense, be looked upon as one of its motor nerves."*

He also opposed the idea that the decelerator response is a sign of exhaustion of the vagus nerve, because:

*"none of the so-called - too powerful - currents are in ordinary cases able to completely arrest the heart's action for more than a few seconds: the influences which prompt the heart to contract become so strong that stimulation of the vagus, be it ever so powerful, fails to prevent it."*

10. Vortrag des Herrn Dr. Wundt „über den Einfluss des Curaregiftes auf Nerven und Muskeln," am 6. Januar 1860.

Der Vortragende unterwarf mit Dr. Schelske, in Fortsetzung einer gemeinsam unternommenen kritisch-experimentellen Untersuchung der verschiedenen für die Muskelirritabilität beigebrachten Beweismittel, über deren ersten Theil, die chemischen Muskelreize betreffend, Dr. Schelske am 5. August v. J. dem Verein Mittheilung gemacht hat, die Einwirkung des Curaregiftes auf Nerven und Muskeln einer ausgedehnteren Untersuchungsreihe. Die Hauptmomente, welche diese berücksichtigte, waren das Verhalten der Reflexbewegungen, sowie der Herzbewegungen mit und ohne Einfluss der Vaguserregung. Als hauptsächlichste Resultate ergeben die Versuche Folgendes:

1) Der Zustand, welchen die Curarevergiftung in den sensibeln und motorischen Nerven hervorruft, ist nicht mit dem Tode identisch; die Reizbarkeit kann sich desshalb, selbst bei den höchsten Graden der Vergiftung, nach kürzerer oder längerer Zeit später wiederherstellen;

2) in allen Fällen von Curarevergiftung giebt es ein Stadium, in welchem die Reflexerregbarkeit gesteigert ist;

3) die Nerven vergifteter Theile bleiben immer bei partieller Vergiftung, nachdem ihre directe Reizbarkeit schon geschwunden ist, noch eine längere Zeit zur Auflösung von Reflexbewegungen geschickt;

4) die Zahl der Herzschläge nimmt nach der Curarevergiftung zu;

5) die Einwirkung des nervus vagus hört in Folge derselben nicht auf, aber erhält einen dem normalen gerade entgegengesetzten Einfluss: tetanische Reizung des Vagus bewirkt nämlich eine Beschleunigung des Herzschlages, die mit dem Wachsen der Reizung zunimmt.

Diese Thatsachen beweisen, dass die Grundannahme, welche man über die Curarewirkung gemacht hat, die Annahme eines Absterbens der Nerven bis in ihre letzten Enden, falsch ist, und dass daher die Curareversuche selber für die Irritabilitätsfrage völlig bedeutungslos sind. Das Curaregift erzeugte in dem Nerven einen Zustand, der von dem des Todes völlig verschieden ist, der nicht einmal einem Zustand transitorischer Erregungslosigkeit entspricht, und der überdies, wie insbesondere die Herzversuche lehren, höchst wahrscheinlich nicht im Hauptstamm des Nerven, sondern nur in den peripherischen Enden desselben im Muskel oder in hier befindlichen Zwischenorganen seinen Sitz hat. Mit dem gelieferten Nachweis, dass die Curareversuche für die selbständige Reizbarkeit der Muskeln von keiner beweisenden Kraft sind, fällt übrigens die nähere Ermittlung der Curarewirkung lediglich der toxikologischen Untersuchung anheim.

Fig. 2 Abstract of the lecture W. Wundt gave on the 6th of january 1860 during a meeting "des Naturhistorisch-medicinischen Vereins zu Heidelberg". The abstract was published in "Heidelberger Jahrbücher der Literatur" vol 11, page 172, 1860.

### 1.1.2. The cardiac accelerator function of the vagus

The vagally mediated accelerator response has been described for the first time by Wundt in 1860 (50) (Fig. 2). In frogs, he and Schelske encountered an **increase** in heart rate during vagal nerve stimulation after their animals had been treated with curare. They assumed that curare produces such an influence upon the vagus that its stimulation no longer slows but quickens the heart:

*"die Zahl der Herzschläge nimmt nach der Curarevergiftung zu und die Einwirkung des nervus vagus hört in Folge derselben nicht auf, aber erhält einem dem normalen gerade entgegengesetzten Einfluss: tetanische Reizung des Vagus bewirkt nämlich eine Beschleunigung des Herzschlages, die mit dem Wachsen der Reizung zunimmt."*

Similar findings were reported by Rutherford, Boehm, Schiff, Dale and colleagues in the frog (4), the ferret (18), the rabbit (4, 42, 44), the cat (4, 15, 18, 44) and the dog (4, 18, 44). Rutherford (42), who used atropine instead of curare to demonstrate the accelerator response to vagal nerve stimulation in the rabbit, rejected the existence of accelerator fibers within the vagus nerve and regarded the acceleration as a result of **indirect** activation of sympathetic nerve fibers:

*"I observed that the laryngeal muscles, oesophagus, stomach, and intestines, were thrown into violent movement by the irritation; and it occurred to me that possibly the excitement of these organs might produce an influence on the cerebrum or medulla oblongata sufficient to excite the cardiac motor nerves."*

In his paper "Ueber paradoxe Vaguswirkungen bei curarisirten Thieren", Boehm (4) turned down the interpretation of Rutherford, because the accelerator effect can still be observed after removing the gastrointestinal system and suggested that the acceleration educed from the stimulation of sympathetic accelerator fibers which run along with the vagal inhibitor fibers within the vagus nerve:

*"Ein näheres Studium der accelerirenden Wirkung des Vagus in der Curarewirkung befestigte in mir die Ueberzeugung, dass man es entschieden mit accelerirenden Nervenfasern zu thun hat, welche im Vagus verlaufen und nur bei vollständiger Lähmung der hemmenden Fasern dieses Nerven ihre Wirkung unmittelbar äussern können. Die Beschleunigung des Herzschlages, welche man durch Vagusreizung erzielt, stimmt in allen*

*Stücken mit den Wirkungen der sympathischen accelerirenden Herznerven überein, welche aus dem Ganglion stellatum hervor zum Herzen treten."*

The view that postganglionic sympathetic nerve fibers within the vagus nerve underlie the acceleration seen during vagal nerve stimulation was adopted by Langley (18). This acceleration, thereby, fitted well in his concept of the Autonomic Nervous System (1898), in which the sympathetic and parasympathetic nervous divisions innervate the heart and have opposite functional effects on the heart rate.

In "Ueber den Ursprung der erregenden Herznerven" (1878), Schiff (44) associated the acceleration described by Wundt with the acceleration he obtained during gentle stimulation of the vagus nerve. He reminded to the fact that he had already described the accelerator influence in 1849, long before Wundt and Schelske observed it for the first time (1859), but that his finding had not been taken serious.

*"Wundt und Schelske ist es später gelungen, bei Fröschen durch einwirkung des Curare die störende Wirkung der Hemmungsfasern zu beseitigen, oder, wie die Verfasser selbst damals glaubten, umzukehren und durch stärkere Reizung Vermehrung der Pulsfrequenz zu erlangen." "Ich habe Wundt und Schelske vorausgesagt, dass man ihre Versuche todschweigen würde, und das hat denn auch die gute deutsche Presse reichlich 15 Jahre lang gethan, und hätte es gerne noch länger fortgesetzt, wenn nicht seitdem paradoxe Nervenwirkungen zu einem Modeartikel geworden wären."*

Schiff (44) rejected the idea of Boehm that sympathetic nerve fibers within the vagus nerve underlie the accelerator response, because he obtained increases in heart rate by stimulating the roots of the vagi in atropinized rabbits, cats and dogs, which proved to him that the acceleration is definitely produced by vagal accelerator fibers. On account of his earlier findings, he suggested that decelerator fibers in the vagus nerve serve a function in modulating the effect of the vagal accelerator fibers:

*"Diese Beobachtungen bewogen mich anzunehmen, dass ausser den Beschleunigungsfasern, deren Gegenwart im Vagus schon früher erwiesen war, im normalen Zustande auch noch eigentliche Hemmungsfasern im Sinne Weber's im Vagus vorhanden sein müssen, welche, beim nicht vergifteten Thiere mitgereizt, verhindern, dass die Energie der Acceleratoren frei hervortrete."*

The existence of vagal accelerator fibers within the vagus nerve was also indicated by the observation of Dale, Laidlaw and Symons in 1910 (18), who found an increase in heart rate in response to vagal nerve stimulation in cats after the stellate, inferior and superior cervical ganglia had been removed. In their paper "A reversed action of the vagus on the mammalian heart", they concluded that:

*"It is clear, therefore, that fibres of the true sympathetic system can play no part in the production of the effect. The accelerator effect being thus undoubtedly due to fibres belonging to the vagus itself. The vagus contains, in addition to its normally predominant inhibitor fibers, others which produce acceleration of the heart-beat."*

Furthermore, these authors reported that acceleration of the heart rate can be revealed by stimulating the left or the right vagus nerve at any point of the vagosympathetic trunk to the separation of the cardiac branches. However, in producing the acceleration, the choice of the animal species and that of the anesthetic is not a matter of indifference. As to the animal, the accelerator effect was most pronounced in cats, while only a moderate accelerator effect was observed in the dog, and they never succeeded in obtaining an accelerator effect in the rabbit. As to the anesthesia, they had varying success in producing the vagal cardiac acceleration during urethane anesthesia, while during paraldehyde anesthesia, which has little depressant action on the heart, an accelerator response was always obtained.

Conclusive evidence for the existence of vagal accelerator fibers was presented in the studies of Jourdan and Nowak in 1934 and 1936 (30, 31), in the study of Kabat in 1940 (33) and in the study of Weiss and Priola in 1972 (49). In the studies of Jourdan and Nowak and that of Kabat, it was demonstrated that sympathectomized dogs with blocked muscarinic receptors, produced a cardiac acceleration when the peripheral end of the cut vagus nerve was stimulated intracranially at its junction with the medulla oblongata. In addition, the vagal cardiac acceleration was demonstrated in cross-circulation experiments: acute ischemia of the isolated head of a dog, which was connected to its trunk only by the vagus nerves and was perfused by a donor dog, produced a decelerator effect before and an accelerator effect after the heart was treated with atropine (30, 31, 33). From their results Jourdan and Nowak (31) concluded:

*"Ainsi c'est donc bien dans la région encéphalo-bulbaire et très vraisemblablement dans la bulbe, et non dans la moelle, que les fibres accélératrices contenues dans le vague ont leur origine."*

Weiss and Priola (49) established that the nucleus ambiguus is the brainstem site for the origin of the vagal cardioaccelerator fibers, because stimulation of the region of the nucleus ambiguus produced a long-lasting acceleration in the presence of atropine. The vagal accelerator pathway is presumably anatomically distinct from the vagal decelerator pathway, because the threshold strength needed to produce acceleration was greater than that needed to produce deceleration (33, 49).

That the acceleration observed during stimulation of the vagosympathetic trunk, is produced by two distinct mechanisms was elucidated by Alter and co-workers in 1973 (1). These authors recognized that the acceleration actually consists of two components; a **fast** component, characterized by a short latency from the onset of the stimulus (2 s) and a fast rate of rise, and a **slow** component, characterized by a relatively long latency (23 s), slow rate of rise and a prolonged recovery time after stimulation had ceased (142 s). The fast component is probably mediated by sympathetic fibers within the vagosympathetic trunk, because it is abolished by $\beta$-adrenoceptor blockade. In contrast, the slow component can be produced in the presence of a $\beta$-adrenoceptor antagonist and does, therefore, not depend on activation of $\beta$-adrenergic receptors.

It has been established that the vagal cardiac acceleration depends on activation of nicotinic receptors, because various authors (1, 19, 47) could not provoke an acceleration by stimulating the vagus nerve in the presence of a nicotinic receptor antagonist.

That the fast component of the acceleration seen during stimulation of the vagosympathetic trunk is actually produced by postganglionic sympathetic nerve fibers within the vagus nerve can be deduced from the results of Antonaccio (2, 3) and Smith (47). These authors obtained an accelerator response upon vagal nerve stimulation in muscarinic and nicotinic receptor blocked dogs, which was abolished after the $\beta$-adrenoceptors were blocked additionally or when the animals had been pretreated with reserpine, a substance which causes depletion of catecholamines in sympathetic nerve terminals (2, 3).

### 1.1.3. Vagal cardiac acceleration versus post-vagal tachycardia

Several investigators reported that the vagal cardiac acceleration can only be demonstrated in the presence of a muscarinic receptor antagonist (18, 33, 49). According to Dale and collaborators (18):

*"the vagus contains, in addition to its normally predominant inhibitor fibres, others which produce acceleration of the heart beat. The function of the paralyzing drug in this case*

*would be discriminating, the accelerator action being revealed by depression of the inhibitor effect which normally masks it."*

Weiss and Priola (49), on the other hand, suggested that the tachycardia produced by vagal accelerator fibers is normally suppressed by the simultaneously activated vagal decelerator fibers.

Despite the fact that vagal cardiac acceleration could be demonstrated in the **presence** of a muscarinic receptor antagonist, vagal cardiac acceleration has often been associated with post-vagal tachycardia, *i.e.* the transient overshoot-tachycardia that follows the bradycardia elicited by vagal nerve stimulation in the **absence** of a muscarinic receptor antagonist (2, 3, 11, 16, 27, 35, 48).

For several reasons, Copen, Cirillo and Vassalle (1968) (16) suggested that vagal cardiac acceleration and post-vagal tachycardia share a single underlying mechanism. Both phenomena are produced by vagal nerve fibers and both tachycardias are abolished in the presence of a nicotinic receptor blocking agent. According to these authors post-vagal tachycardia and vagal cardiac acceleration result from excitation of parasympathetic nerve fibers. Upon excitation, these fibers release acetylcholine. Subsequently, acetylcholine stimulates nicotinic receptors on cardiac chromaffin cells and thereby causing the liberation of catecholamines. Later on, it was established that vagal cardiac acceleration (1, 2, 19) and post-vagal tachycardia (11, 27, 35) are not mediated by catecholamines, because both tachycardias are found in the presence of a $\beta$-adrenoceptor antagonist and in reserpine-pretreated animals as well.

On the other hand, Burke and Calaresu (1972) (11) and Iano, Levy and Lee (1973) (27) rejected the hypothesis that the two phenomena share the underlying mechanism, because post-vagal tachycardia needs functional muscarinic receptors, while vagal cardiac acceleration is only observed when muscarinic receptors are blocked. According to Burke and Calaresu (11), vagal cardiac acceleration is a non-physiological phenomenon and:

*"should be considered an artifact of vagal stimulation and not a physiological mechanism of post-vagal tachycardia,"* because *"a tachycardia could be elicited in atropinized animals only by increasing the intensity of the stimulus to the vagus nerve."*

Whether post-vagal tachycardia and vagal cardiac acceleration should indeed be considered as distinct phenomena with different underlying mechanisms is discussed further in the general discussion.

### 1.1.4. Reflex activation of the vagal cardiac accelerator system

As outlined in 1.1.2. vagal cardiac acceleration has been produced by stimulation of vagal efferent nerve fibers, by stimulation of the origin of these fibers in the brainstem, and by inducing brain ischemia. In attempting to elucidate the physiological relevance of the vagal cardiac accelerator system, Brouha, Cannon and Dill (5) and Kabat (33) tried to excite the vagal accelerator fibers reflexly by stimulating afferent fibers. Their attempts failed.

Kabat (33) reported that when he, in the **absence** of atropine, stimulated the vagal **afferent** nerves in sympathectomized dogs, he always obtained a deceleration, while when he stimulated the vagal **afferent** nerves in the **presence** of atropine he never observed a change in heart rate, despite the fact that stimulation of the vagal **efferent** nerves always led to an increase in heart rate in these experiments. The findings of Kabat are in agreement with those of Brouha, Dill and Nowak (7), who also did not obtain an accelerator response to vagal afferent nerve stimulation. In contrast, Jourdan and Morin (32) claimed that, in the presence of atropine, they observed a vagal cardiac acceleration when they stimulated the central end of the superior laryngeal nerve. On account of the fact that these authors performed only partial sympathectomy, Kabat (33) asserted that this acceleration was presumably mediated by sympathetic nerve fibers instead of being produced by the vagal cardiac accelerator system.

Kabat (33) and Brouha et al. (5) also obtained negative results when they tried to reflexly activate the vagal cardiac accelerator fibers by inducing a decrease in the blood pressure. Copen et al. (16), however, point to the fact that these authors should have increased instead of decreased the arterial blood pressure in order to activate the vagal cardiac accelerator system, because the vagal tone increases when the blood pressure is elevated.

Since the activity of the vagal cardiac accelerator system had only been demonstrated by means of artificial procedures and none of the attempts to reflexly activate the vagal cardiac accelerator system succeeded, its relevance and even its existence remained a disputed issue.

## 1.2. Inherent vagal cardiac acceleration

### 1.2.1. Vagal cardiac accelerator response to physiological stimuli

A first indication of the significance of the vagal cardiac accelerator system has been found by Brouha et al. (5, 6, 7), who observed that sympathectomized, adrenalectomized dogs responded to exercise and emotional excitement with an increase in heart rate to a level in excess of 200 beats/min, which is well above the heart rate they encountered after complete denervation of the heart, *i.e.* 130 beats/min. According to these authors, the acceleration during exercise and emotional excitement resulted from a reduction of the vagal cardiac decelerator tone and an augmentation of the vagal cardiac accelerator tone. The vagal cardiac accelerator system probably comes into play when the sympathetic accelerator control is lost and it serves a function similar to that of the sympathetic nervous division. Hodes (25) confirmed that the acceleration during exercise has a vagal origin. In his experiments, he demonstrated that sympathectomized cats responded to exercise with an increase in heart rate only when the vagi were intact.

### 1.2.2. Vagal cardiac acceleration observed following atropine treatment

Brouha, Cannon and Dill (5) were also the first to recognize that the inherent vagal cardiac accelerator activity can be demonstrated by injecting atropine into sympathectomized dogs:

*"Injection of atropine sulphate into unanaesthetized, sympathectomized dogs with the vagi intact is followed by an acceleration of the heart, the rate reaching 210-230 beats per min. Under urethane anaesthesia after section of both vagi in the neck, the heart rate stabilizes at about 130 beats per min., and the injection of the same dose of atropine produces no effect: the heart rate remains constant. If the vagi are now stimulated, the heart rate increases considerably. In one dog the rate reached 230 beats per min., the maximum that had been previously obtained on injecting atropine when unanaesthetized, the maximum also that was obtained by intense exercise of long duration. From this we may conclude that double vagotomy suppresses some accelerator mechanism which comes into play under the influence of atropine when the vagi are intact."*

The vagal origin of the cardiac acceleration was confirmed in several studies in which no acceleration was obtained following the administration of atropine into

sympathectomized, vagotomized dogs (5, 19, 20). More recently, Brunsting et al. (8, 9) demonstrated in β-adrenoceptor and muscarinic receptor blocked dogs that the inherent vagal cardiac acceleration disappeared when the vagi were transected or when a nicotinic receptor antagonist was administered. Rigel et al. (39) reported that insignificant increases in heart rate were encountered when an antimuscarinic agent was injected into β-adrenoceptor blocked conscious dogs of which the vagus nerves were cooled. After rewarming the vagi the heart rate increased to a level of 193 beats/min. After cooling the vagus nerves, the vagal cardiac acceleration disappeared and the heart rate stabilized at the same level as observed before rewarming the vagus nerves (about 130 beats/min). To quantify the vagal cardiac acceleration, which they designated "excess tachycardia", they subtracted the denervated heart rate, *i.e.* the heart rate after β-adrenoceptor and muscarinic receptor blockade in combination with vagal nerve cooling, from the heart rate observed after combined β-adrenoceptor and muscarinic receptor blockade.

As to the cause of the vagal cardiac acceleration, Brouha and Nowak (6) discussed the suggestion of Moore and Cannon (38) that the vagally induced acceleration is in fact an atropine-acceleration (atropine-tachycardia), since it is produced by the central stimulant action of atropine on vagal nerve activity. Brouha and Nowak (6), however, suggested that the acceleration resulted from the unmasking of a vagal accelerator action after elimination of the vagal decelerator action:

*"One might suppose that an increase of the heart rate to 200 beats/min. or more represents a positive stimulus due to the central action of atropine. This mode of action of atropine would, of necessity, be dual: inhibitory on the cardiac vagal parasympathetic endings, and cerebral excitatory on the mesencephalic origin of the vagal accelerator fibres. It would be simpler to conceive the total atropine-acceleration as due to a single parasympathetic inhibitory effect, the acceleration being due to release of a potential accelerator mechanism. In such a case the phenomenon of atropine with preservation of the accelerator effect represents another example of the selective action of a drug on a mixed nerve."*

On account of the fact that atropine does not produce an acceleration in dogs which are anesthetized with sodium pentobarbital or morphine-chloralose, and the fact that atropine does not affect the heart rate of vagotomized dogs in the conscious state, Donald et al. (19) revived the hypothesis that the acceleration is due to the central stimulant action of atropine on vagal nerve activity. However, Chassaing et al. (12) rendered this idea quite improbable by showing that the injection of methylatropine or

methylscopolamine evoked a similar accelerator response in spite of the fact that these compounds, unlike atropine, hardly cross the blood-brain barrier. Chassaing et al. (12), therefore, postulated that the atropine-acceleration is brought about by a peripheral mechanism:

*"Quoi qu'il en soit, puisque des vagolytiques et des bêta-bloquants qui ne franchissent pas la barrière hémato-méningée donnent des résultats comparables à ceux de l'atropine et du propranolol, c'est bien dans un mécanisme périphérique qu'il faut chercher l'origine des effets paradoxaux constatés."*

Based on similar finding as Chassaing et al. (12), Schuil et al. (45) adopted the hypothesis of Brouha and Nowak that vagal cardiac acceleration is the unmasking of a vagal cardiac accelerator tone after the loss of vagal decelerator action.

The magnitude of the vagal cardiac accelerator activity which is observed after the injection of an antimuscarinic agent has been reported to vary greatly from one dog to another (13, 39, 46). The vagal cardiac accelerator activity may even be absent in some dogs. Rigel et al. (39) reported that one of their six dogs consistently exhibited no vagal cardiac acceleration, Schuil (46) disclosed that in 11 of his 71 dogs no vagal cardiac acceleration was observed, and Hill et al. (23) excluded three of the fifteen dogs from the results presented, because these animals lacked a vagal cardiac accelerator activity.

The vagal cardiac acceleration which is induced by electric vagal nerve stimulation has been reported to vary considerably between animals (4, 33, 44). Boehm (4) wrote already in 1875:

*"Wir haben diese pulsbeschleunigende Wirkung des Vagusreizes bei Katzen und Hunden auf das Unzweideutigste constatiren können. Die Ursachen, warum sie in einzelnen Fällen ausbleibt, sind mir dunkel geblieben."*

and Schiff (44) remarked in 1878:

*"Diese Versuche waren theilweise an Hunden, besonders aber an Katzen gemacht worden und abgesehen von einigen Thieren, bei denen jede Wirkung fehlte oder zweifelhaft blieb, hatte ich damals an zwei und zwanzig vergifteten Katzen durch Reizung des Vagus namhafte Pulsvermehrung erzielt."*

Although the factor underlying the interindividual variation in vagal cardiac acceleration has not been elucidated yet, Kabat (33) surmised that the sympathetic nervous division is somehow involved in the control of the accelerator response to vagal nerve stimulation:

*"Thus Jourdan and Nowak (1936) had no difficulty in demonstrating accelerator fibers on stimulation of the vagus nerve because they had previously performed a bilateral stellectomy. On the other hand, Hering (1924) and Kabat (1937, in a preliminary note on some of the experiments reported here) were reluctant to admit the existence of vagal cardio-accelerator fibers because the results obtained were largely negative and whatever acceleration was observed was slight. The relative ineffectiveness of stimulation of the vago-sympathetic nerve in these experiments may be accounted for by the fact that the sympathetic innervation was left intact."*

### 1.2.3. Transient nature of the vagal cardiac acceleration

Brouha et al. (5) demonstrated that the heart rate of sympathectomized dogs which perform light exercise increases within a few minutes to a level of 120-140 beats/min, during average exercise to about 160 beats/min, and during intense exercise to about 200 beats/min. Furthermore, they recognized that when the exercise becomes more vigorous or more prolonged the heart rate progressively increases to even higher levels. In contrast, Brouha and Nowak (6) noticed that in sympathectomized dogs, the vagal cardiac acceleration which is observed after the injection of atropine is transient in nature:

*"The maximal cardiac acceleration was observed 1-10 min. after intravenous, and 15-30 min. after subcutaneous administration. The accelerator effect diminished after these time limits, the rate returning to normal in about 2 h."*

Donald et al. (19) demonstrated that the vagal cardiac acceleration is also transient when the animals were either treated with the $\beta$-adrenoceptor antagonist propranolol or with reserpine instead of being sympathectomized. Chassaing et al. (13) excluded the possibility that the vagal cardiac acceleration decreases as a function of the decrease in circulating concentration of atropine, because when atropine was administered by infusion, the progressive decrease in the acceleration was accompanied by an increase in the concentration of atropine. The mechanism underlying the progressive decrease in vagal cardiac acceleration is still not known, but Chassaing et al. (13) presumed that the

sympathetic nervous division is involved in this decrease, because the vagal cardiac acceleration lasts much longer in sympathectomized than in β-adrenoceptor blocked dogs.

Rigel et al. (39) reported that in β-adrenoceptor blocked conscious dogs, the vagal cardiac acceleration spontaneously declined despite a constant infusion of the antimuscarinic agent and repeated injections of the original bolus dose. The vagal cardiac acceleration

*"typically declined to 50 and 20% of the maximum levels after 30 and 70 min, respectively, and, after about 2 h, the heart rate usually stabilized to within a few beats per minute of the heart rate level that had been observed during vagal cooling."*

In the same study, Rigel et al. (39) noticed that during the spontaneous decline of the vagal cardiac acceleration, it was possible to reflexly modulate the accelerator activity by altering the arterial blood pressure. A rise in blood pressure induced an enhancement of the vagal cardiac accelerator activity. Conversely, a fall in blood pressure induced an attenuation of the vagal cardiac accelerator activity. That the vagal cardiac accelerator activity was altered through a reflex, was inferred from the finding that the heart rate did not respond to the administration of the vasoactive agents phenylephrine and sodium-nitroprusside nor to the resulting changes in blood pressure when the vagus nerves were cooled. These authors, thereby, for the first time indicated a role of the vagal cardiac accelerator system in the regulation of the arterial blood pressure. However, when the vagal cardiac acceleration had disappeared, approximately 2 h after the injection of the muscarinic receptor antagonist, the ability to reactivate the vagal cardiac accelerator system by increasing the arterial blood pressure was lost.

### 1.2.4. The role of anesthetics in the study of the vagal cardiac accelerator system

One of the major reasons that the existence of the vagal cardiac accelerator system did not become commonly acknowledged, is that in most experiments, especially those in which surgical procedures were performed, anesthetics were used. Various authors have reported that hardly any vagal cardiac accelerator activity is observed when atropine is injected into animals anesthetized with either pentobarbital (6, 9, 19), chloralose (14, 19) or methoxyflurane (8). From this finding, Donald et al. (19) deduced that anesthetics, through their central depressant action, inhibit the central stimulant action of atropine and thereby prevent the production of an acceleration. Others suggested the vagal cardiac accelerator activity is considerably reduced due to the vagolytic action of

the anesthetics (9, 39). In agreement with the latter hypothesis is the finding that neuroleptanesthetized dogs exhibit a vagal cardiac acceleration that is comparable to that found in dogs in the conscious state (8) and that neuroleptanesthesia, which in the dog is induced by means of a combination of the neuroleptic fluanisone and the analgetic fentanyl, does not possess vagolytic properties. Moreover, neuroleptanesthesia is proposed to be suitable, because vagal cardiac acceleration has then the same transient nature as observed in conscious dogs. With the use of neuroleptanesthesia, Brunsting et al. (9, 10) and Schuil et al. (45, 46) were able to confirm the vagal origin of the acceleration by showing that the vagal cardiac acceleration, which became manifest after blocking muscarinic receptors in $\beta$-adrenoceptor blocked dogs, disappeared when the vagus nerves were sectioned. Furthermore they confirmed the hypothesis of Donald et al. (19) that a ganglionic nicotinic receptor is involved in the vagal accelerator pathway, by showing that the vagal cardiac acceleration disappeared after a nicotinic receptor antagonist was administered in $\beta$-adrenoceptor and muscarinic receptor blocked dogs (8, 9, 45, 46). On the other hand the relationship between vagal cardiac acceleration and arterial blood pressure, as established in conscious dogs (39), could not be established in $\beta$-adrenergic and muscarinic receptor blocked dogs which were in neuroleptanesthesia (46).

### 1.3. The terminal transmitter in the vagal cardiac accelerator pathway

Traditionally the vagus nerve is considered within the concept of the autonomic nervous system to exert its actions through the release of acetylcholine. However, the substance that is released from the vagal nerve terminals and that produces an acceleration has not been identified yet. After it was established that the vagal cardiac acceleration is not produced by sympathetic nerve fibers that run along with vagal nerve fibers within the vagosympathetic trunk, various authors presumed that the acceleration is produced by catecholamines released from either cardiac chromaffin tissue (2, 28) or intracardiac adrenergic nervous structures (26, 33, 47) and that the catecholamine release is under control of the vagal nervous division. In this connection Kabat (33) remarked:

*"if this mechanism is substantiated by further investigation, then one is presented with yet another example of the presence of adrenergic and cholinergic autonomic nerve fibers in the same anatomic nerve. It is evident that the old anatomic subdivision of the autonomic nervous system into sympathetic and parasympathetic systems is rapidly losing its usefulness and must give way to a more fundamental functional classification."*

After adrenergic receptor antagonists and reserpine became available, the possible role of catecholamines in the vagal cardiac acceleration was ruled out, because an inherent vagal cardiac acceleration was also encountered in atropinized dogs which were either pretreated with reserpine (13, 19) or treated with a $\beta$-adrenoceptor antagonist (1, 13, 19), or the combination of a $\beta$-adrenoceptor and an $\alpha$-adrenoceptor antagonist (8, 9, 45).

Several authors suggested that acetylcholine caused the vagal cardiac acceleration through nicotinic receptors which are localized on the membranes of the sinus node cells (16, 39). This hypothesis is supported by the finding that acetylcholine produced an acceleration in atropinized isolated heart preparations and in intact atropinized animals (21, 22, 26, 37) and by the finding of nicotinic receptors on sinus node cells and that activation of these receptors by acetylcholine induced an effect opposite to that following muscarinic receptor activation (34). Donald et al. (19), however, suggested that acetylcholine increases the heart rate by activating sympathetic nerve fibers, because the increase is only obtained in the absence of a $\beta$-adrenoceptor antagonist. When his animals were either treated with a $\beta$-adrenoceptor antagonist or pretreated with reserpine, he never observed a chronotropic effect following acetylcholine administration. Also James and Nadeau (29) turned down acetylcholine as the terminal transmitter in the vagal cardiac accelerator pathway, because they never observed an acceleration when acetylcholine was injected directly into the sinus node artery of the atropinized, $\beta$-adrenoceptor blocked heart, while in the same experiments vagal nerve stimulation produced an acceleration.

Although not being the terminal transmitter, acetylcholine does seem to be involved in the production of vagal cardiac acceleration, since vagal nerve stimulation does not result in an acceleration in the presence of a nicotinic receptor antagonist (1, 16, 19,47). Donald et al. (19), who showed that the administration of atropine did not produce an acceleration in nicotinic receptor blocked conscious animals, suggested that a ganglionic nicotinic receptor is involved in the vagal cardiac accelerator pathway. The same conclusion was drawn by Brunsting et al. (9) on account of the finding that the vagal cardiac acceleration, as observed in $\beta$-adrenergic and muscarinic receptor blocked neuroleptanesthetized dogs, disappeared following the administration of hexamethonium (8, 9). In this connection, these authors emphasized that, in the determination of the denervated heart rate, the conventional pharmacological approach to eliminate the autonomic nervous control, *i.e.* the injection of a $\beta$-adrenergic and a muscarinic receptor antagonist, is insufficient, since a nicotinic receptor antagonist is required in order to eliminate the vagal cardiac accelerator influence.

Rigel et al. (39) suggested that histamine might be the terminal transmitter in the vagal cardiac accelerator system, because the sinus node is rich in histamine-storing mast cells and histamine exerts a positive chronotropic effect that is not blocked by $\beta$-adrenoceptor antagonists. This hypothesis was supported by Brunsting et al. (10) who found that histamine receptor antagonists reduce the stimulation-induced vagal cardiac acceleration. However, they advanced the notion that multiple mediators are involved in the vagal cardiac acceleration, because the serotonin receptor antagonists cyproheptadine and ketanserin and the histamine receptor antagonists promethazine and cimetidine, all to a different extent, reduce the stimulation-induced vagal cardiac acceleration. In another study, Rigel and Katona (40) turned down the involvement of histamine, because a 30-fold greater dose of a histamine receptor antagonist was needed to reduce the vagal cardiac acceleration than to block the chronotropic effect of histamine.

It has recently been established that the autonomic control of the heart is not attributed solely to cholinergic and (nor)adrenergic nerve fibers, but that the heart is innervated by peptidergic fibers as well, and that neuropeptides are frequently co-stored and co-released along with acetylcholine and norepinephrine in parasympathetic and sympathetic nerve terminals, respectively (17). It is, therefore, possible that the vagal cardiac acceleration represents the action of one or more recently recognized peptidergic neurotransmitters (17). Strong arguments for a peptidergic involvement in the vagal cardiac acceleration have already been put forward. Firstly, neuropeptides have been found co-localized with acetylcholine within the same vagal nerve terminal. Secondly, the release of neuropeptides and the production of a stimulation-induced vagal cardiac acceleration are both most pronounced when the nerves are stimulated at high frequencies, *i.e.* between 10-30 Hz (23, 24). In this respect, it should be remembered that, already in 1860, Wundt (50) remarked that:

*"tetanische Reizung des Vagus bewirkt nämlich eine Beschleunigung des Herzschlages, die mit dem Wachsen der Reizung zunimmt."*

and that Kabat (33) and Weiss and Priola (49) suggested that the vagal accelerator and decelerator pathways are anatomically distinct, because the threshold strength needed to produce acceleration was greater than needed to produce deceleration. Burke and Calaresu (11) also considered the vagal cardiac acceleration of no physiological meaning just because the vagal cardiac acceleration can be elicited in atropinized animals only by increasing the intensity of the stimulus to the vagus nerve. Thirdly, it has been established that the action of neuropeptides is prolonged, while it has also been described that the

accelerator response to vagal nerve stimulation endures after the stimulation had ceased (1). Fourthly, in searching for potential candidates for the terminal transmitter in the vagal cardiac accelerator system, Rigel (41) examined the positive chronotropic effects of several neuropeptides in β-adrenergic and muscarinic receptor blocked, stellectomized, vagotomized, pentobarbital-anesthetized dogs and found that some neuropeptides produce a positive chronotropic effect that is comparable to the stimulation-induced vagal cardiac accelerator response. These authors, therefore, suggested that neuropeptides might be involved in the non-adrenergic, non-cholinergic control of the heart rate.

## 1.4. Aim of the study

The principal aim of the present study was to extend the knowledge of the vagal cardiac accelerator system. In all studies, the inherent vagal cardiac accelerator activity was uncovered by blocking the β-adrenergic receptors with the aselective β-adrenoceptor antagonist timolol and the muscarinic receptors with the aselective muscarinic receptor antagonist methylatropine. **Chapter 2** was designed to investigate the role of peripheral factors in the transient nature of the inherent vagal cardiac acceleration. First, the rate of decline of the inherent vagal cardiac acceleration was studied in neuroleptanesthetized dogs. Subsequently, the accelerator response to vagal nerve stimulation was examined in the case that vagotomy was performed 10 min after the administration of methylatropine, *i.e.* before the inherent vagal cardiac acceleration started to decline and, in a second series of experiments, when vagotomy was performed about 3 h after the administration of atropine, *i.e.* after the vagal cardiac acceleration had almost completely disappeared. In **chapter 3** the heart rate and blood pressure responses to successive β-adrenoceptor and muscarinic receptor blockade were compared between dogs in neuroleptanesthesia and in the conscious state. Furthermore, the influence of preceding α-adrenoceptor blockade on the heart rate and blood pressure responses to successive β-adrenoceptor and muscarinic receptor blockade was compared in the neuroleptanesthetized and conscious state. In **chapter 4** the possible involvement of the vagal cardiac accelerator system in the baroreceptor reflex mechanism was studied by examining the influence of pharmacologically induced blood pressure alterations on the vagal cardiac accelerator activity.

In order to identify the terminal transmitter in the vagal cardiac accelerator system the chronotropic effects of various substances, acetylcholine, histamine and vasoactive intestinal polypeptide (VIP), were examined in β-adrenoceptor and muscarinic receptor blocked conscious dogs. In order to circumvent the reflex changes in vagal cardiac accelerator activity induced by the drug-induced blood pressure alterations (the

mechanism that is described in chapter 4), we developed a cannula-catheter system that can be chronically implanted in the coronary artery of a dog. This device enabled us to inject substances directly into the blood supply of the sinus node of conscious animals. The cannula-catheter system and the technique of cannulation are described in **chapter 5**. In the experiments described in **chapter 6** the chronotropic properties of VIP were studied with VIP injected either intravenously or intracoronary into β-adrenoceptor and muscarinic receptor blocked conscious dogs. In order to find conclusive evidence that VIP is the terminal transmitter in the vagal cardiac accelerator pathway, the effect of the VIP receptor antagonists [D-p-Cl-Phe$^{6}$,Leu$^{17}$]-VIP and [Lys$^{1}$,Pro$^{2,5}$,Leu$^{17}$]-VIP on the prevailing vagal cardiac accelerator activity, the reflex vagal cardiac accelerator response to a rise in blood pressure, and the VIP-induced heart rate increase were examined.

Although the vagal cardiac accelerator response to vagal nerve stimulation was encountered in various species, including frog, fret, rabbit, cat and dog, the inherent vagal cardiac acceleration, as becomes manifest following the administration of a muscarinic receptor antagonist in β-adrenoceptor blocked animals, has only been recognized in dogs. In order to find proof that the vagal cardiac accelerator system is also present in other animals than the dog, we blocked the β-adrenergic and muscarinic receptors in rats and lambs (**chapter 7**). In addition, we studied the influence of pharmacologically induced alterations in blood pressure on the heart rate in the absence and in the presence of methylatropine in β-adrenoceptor blocked rats and lambs.

In the general discussion (**chapter 8**) the results of the different studies described in this thesis are reviewed. On the basis of the findings, a general model is presented, which describes the mechanisms underlying the vagal cardiac acceleration and the relation between the vagal cardiac accelerator system and the arterial blood pressure.

## 1.5. References


1. Alter III WA, Weiss GK, Priola DV (1973) Vagally induced tachycardia in atropinized dogs: effect of β-adrenergic blockade. *Eur J Pharmacol* 24:329-333
2. Antonaccio MJ, Robson RD (1974) Pharmacological alterations of vagally induced tachycardia in anesthetized dogs. *Eur J Pharmacol* 25:19-28
3. Antonaccio MJ (1974) Paradoxical vagal tachycardia in ganglion-blocked anesthetized dogs. *Eur J Pharmacol* 28:59-65
4. Boehm R (1875) Ueber paradoxe Vaguswirkungen bei curarisirten Thieren; Beiträge zur Kenntniss der Physiologie der Herznerven und der Pharmakologie des Curare. *Arch Exp Pathol Pharmakol* 4:351-373
5. Brouha L, Cannon WB, Dill DB (1936) The heart rate of the sympathectomized dog in rest and exercise. *J Physiol* 87:345-359
6. Brouha L, Nowak SJG (1939) The role of the vagus in the cardio-accelerator action of atropine in sympathectomized dogs. *J Physiol* 95:439-453
7. Brouha L, Nowak SJG, Dill DB (1939) The role of the vagus in the cardio-accelerator action of muscular exercise and emotion in sympathectomized dogs. *J Physiol* 95:454-463
8. Brunsting JR, Bennekers JH, Schuil HA, Zijlstra WG (1979) Incomplete cardiac vagal blockade with atropine in the anesthetized dog. *Pflügers Arch* 381:293-295
9. Brunsting JR, Schuil HA, Zijlstra WG (1983) Intrinsic heart rate in the dog determined by pharmacologic denervation. *Am J Physiol* 245:H592-H597
10. Brunsting JR, Patberg WR, Schuil HA, Zijlstra WG (1985) Effect of autacoid antagonists on vagal cardioacceleration in the dog. *J Physiol* 36:39
11. Burke GH, Calaresu FR (1972) An experimental analysis of the tachycardia that follows vagal stimulation. *J Physiol* 226:491-510
12. Chassaing C, Duchêne-Marullaz P, Moins N, Combre A (1976) Effets paradoxaux de propranolol et d'atropine sur la fréquence cardiaque du chien non narcosé. *J Pharmacol (Paris)* 5:609-618
13. Chassaing C, Godeneche D, Boucher M, Duchêne-Marullaz P (1979) A comparison of changes in atropine-induced tachycardia and atropine concentration in conscious dogs. *Eur J Pharmacol* 58:433-441
14. Chassaing C, Boucher M, Breysse C, Duchêne-Marullaz P (1992) Contribution of vagal blockade to the tachycardia induced by the antimuscarinic agent atropine and pirenzepine. *J Auton Pharmacol* 12:359-368
15. Chiang TS, Leaders FE (1966) Cardiostimulatory responses to vagal stimulation, nicotine, and tyramine. *Am J Physiol* 211:1443-1446
16. Copen DL, Cirillo DP, Vassalle M (1968) Tachycardia following vagal stimulation. *Am J Physiol* 215:696-703
17. Corr L (1992) Neuropeptides and the conduction system of the heart. *Int J Cardiol* 35:1-12
18. Dale HH, Laidlaw PP, Symons CT (1910) A reversed action of the vagus on the mammalian heart. *J Physiol* 41:1-18
19. Donald DE, Samueloff SL, Ferguson D (1967) Mechanisms of tachycardia caused by atropine in conscious dogs. *Am J Physiol* 212:901-910
20. Freeman NF, Page lH (1937) Hypertension produced by constriction of the renal artery in sympathectomized dogs. *Am Heart J* 14:405-414

21. Haney HF, Lindgren AJ (1945) The effect of acetylcholine on the atropinized, denervated heart. *Am J Physiol* 145:177-180
22. Heymans C, Bennati D (1949) Mécanisme de la tachycardie par l'acétylcholine. *Arch Int Pharmacodyn Ther* 79:486-489
23. Hill MRS, Wallick DW, Martin PJ, Levy MN (1990) Excess tachycardia: an anesthetized preparation and its stimulation frequency response. *Proc Annu Int Conf IEEE Eng Med Biol Soc* 12:757-758
24. Hill MRS, Wallick DW, Martin PJ, Levy MN (1993) Frequency dependence of vasoactive intestinal polypeptide release and vagally induced tachycardia in the canine heart. *J Auton Nerv Syst* 43:117-122
25. Hodes R (1939) Exercise in the sympathectomized cat. *Am J Physiol* 126:171-179
26. Hoffmann F, Hoffmann EJ, Middleton S, Talesnik J (1945) The stimulating effect of acetylcholine on the mammalian heart and the liberation of an epinephrine-like substance by the isolated heart. *Am J Physiol* 144:189-198
27. Iano TL, Levy MN, Lee MH (1973) An accelerator component of the parasympathetic control of heart rate. *Am J Physiol* 224:997-1005
28. Jacobowitz D (1967) Histochemical studies of the relationship of chromaffin cells and adrenergic nerve fibers to the cardiac ganglia of several species. *J Pharmacol Exp Ther* 158:227-240
29. James TN, Nadeau RA (1963) Sinus bradycardia during injections directly into the sinus node artery. *Am J Physiol* 204:9-15
30. Jourdan F, Nowak SJG (1934) Les fibres cardio-accélératrices dans le nerf pneumogastrique du chien; leur origine et leur trajet. *C R Soc Biol* 117:234-239
31. Jourdan F, Nowak SJG (1936) Étude expérimentale chez le chien des fibres cardio-accélératrices du vague. *Arch Int Pharmacodyn Ther* 53:121-135
32. Jourdan F, Morin G (1936) Mise en jeu réflexe des fibres accélératrices cardiaques du nerf pneumogastrique. *C R Soc Biol* 121:49-51
33. Kabat H (1940) The cardio-accelerator fibers in the vagus nerve of the dog. *Am J Physiol* 128: 246-257
34. Lipsius SL, Vassalle M (1977) Effects of acetylcholine on potassium movements in the guinea pig sinus node. *J Pharmacol Exp Ther* 201:669-677
35. Loeb JM, Vassalle M (1979) Adrenergic mechanisms in postvagal tachycardia. *J Pharmacol Exp Ther* 210:56-63
36. Long DM, Truex RC, Friedmann KR, Olsen AK, Phillips SJ (1958) Heart rate of the dog following autonomic denervation. *Anat Rec* 130:73-89
37. McDowall RJS (1946) The stimulation action of acetylcholine on the heart. *J Physiol* 104:392-403
38. Moore RM, Cannon WB (1930) The heart rate of unanesthetized normal, vagotomized, and sympathectomized cats as affected by atropine and ergotoxine. *Am J Physiol* 94:201-208
39. Rigel DF, Lipson D, Katona PG (1984) Excess tachycardia: heart rate after antimuscarinic agents in conscious dogs. *Am J Physiol* 246:H168-H173
40. Rigel DF, Katona PG (1986) Effects of antihistamines and local anesthetics on excess tachycardia in conscious dogs. *J Pharmacol Exp Ther* 238:367-371
41. Rigel DF (1988) Effects of neuropeptides on heart rate in dogs: comparison of VIP, PHI, NPY, CGRP and NT. *Am J Physiol* 255:H311-H317
42. Rutherford W, (1869) Influence of the vagus upon the vascular system. *J Anat Physiol* 3:402-416
43. Schiff M (1849) Experimentelle Untersuchungen über die Nerven des Herzens. *Arch Physiol*

8:166-234

44. Schiff M (1878) Ueber den Ursprung der erregenden Herznerven. *Arch Gesam Physiol* 18: 172-235
45. Schuil HA, Brunsting JR, van der Molen H, Zijlstra WG (1981) Cardioacceleratory effect of muscarinic blocking agents in the dog. *Eur J Pharmacol* 69:229-233
46. Schuil HA (1987) *Vagale cardio-acceleratie en denervatie van het hondehart*. Thesis, University of Groningen, Van Denderen, Groningen, The Netherlands
47. Smith DC (1970) Synaptic sites in sympathetic and vagal cardioaccelerator nerves of the dog. *Am J Physiol* 218:1618-1623
48. Vassalle M, Mandel WJ, Holder MS (1970) Catecholamine stores under vagal control. *Am J Physiol* 218:115-123
49. Weiss GK, Priola DV (1972) Brainstem sites for activation of vagal cardioaccelerator fibers in the dog. *Am J Physiol* 223:300-304
50. Wundt W (1860) Über den Einfluss des Curaregiftes auf Nerven und Muskeln. *Verhandlungen des naturhistorisch-medicinischen Vereins zu Heidelberg, Heidelberger Jahrbücher der Literatur* 11:172

# 2

## MECHANISM OF THE DECLINE IN VAGAL CARDIAC ACCELERATION IN DOGS IN NEUROLEPTANESTHESIA

**Abstract**

Muscarinic receptor blockade in β-adrenoceptor blocked dogs in neuroleptanesthesia reveals the accelerator influence of the vagal cardiac accelerator system; the heart rate (HR) reaches a maximum level of approximately 200 beats/min within 10 min, which is far in excess of the denervated HR. Subsequently, the vagal cardiac acceleration (VCA) spontaneously declines. Experiments were designed to settle whether the decline in VCA is due to a decrease in peripheral responsiveness and / or a decrease in vagal tone. The decline in VCA was assessed in one group of dogs ($n=12$) over a period of 170 min, after which ganglionic nicotinic receptor blockade (exp. 1) or vagotomy (exp. 2) was carried out. The maximum "inherent VCA", defined as the difference between maximum HR after muscarinic receptor blockade and HR after nicotinic receptor blockade or vagotomy, was $68\pm10$ and $64\pm11$ beats/min, respectively. The VCA activity declined to 75, 50 and 25% maximum levels after $23\pm7$, $42\pm8$ and $127\pm18$ min (exp. 1) or $21\pm4$, $51\pm12$ and $113\pm20$ min (exp. 2). The maximum HR in response to electric vagal nerve stimulation (maximum "stimulation VCA") was established according to two scenarios in two groups of dogs matched for maximum "inherent VCA" ($n=7$). In the 0-min (exp. 3) and 170-min (exp. 4) scenario vagotomy was carried out 10 and 180 min after the muscarinic receptor blockade. Expressed as percentage of the maximum "inherent VCA", the maximum "stimulation VCA" was $116\pm7$ and $41\pm8$%, respectively. This implies that the peripheral neuroeffector responsiveness declined by 75% over the given time period. The rate of the spontaneous decline of "inherent VCA" over the same period was 77% (exp. 4) and did, thereby, not differ from the rate of decline of the maximum "stimulation VCA". This result indicates that the spontaneous decline in VCA activity is due to a decrease in response of the peripheral neuroeffector system.

## Introduction

It has been well documented that administration of a muscarinic receptor blocking agent in sympathectomized, reserpinized or adrenoceptor blocked dogs elicits a cardiac acceleration which is non-adrenergic, non-cholinergic mediated and which can be abolished by vagotomy (2, 5, 7, 11, 13, 25, 28, 29). Brouha and Nowak (3) were the first to notice the transient nature of the vagal cardiac acceleration (VCA). After a bolus injection of atropine in sympathectomized conscious dogs, the heart rate reached a maximum within 10 min and slowly decreased during the succeeding hours. Later on, Donald et al. (11) recognized that VCA diminished spontaneously in muscarinic receptor blocked conscious dogs which were either treated with a $\beta$-adrenoceptor antagonist or reserpine instead of being sympathectomized. More recently, Rigel et al. (25) observed that VCA declined to 50% of the maximum level in 30 min and that after 120 min the heart rate stabilized within a few beats of the denervated heart rate. It was ruled out that the VCA decreased as a function of the atropine washout, because during infusion of atropine with a progressive rise in its blood concentration the same decrease in VCA was observed (8). In a previous study, we reported a similar decrease in VCA in neuroleptanesthetized dogs (4). Moreover we established that VCA can be abolished, besides by vagotomy, by blocking ganglionic nicotinic receptors. By blocking the nicotinic receptors in $\beta$-adrenoceptor and muscarinic receptor blocked dogs, we were able to quantify the vagal cardiac accelerator influence (4, 28) and to determine the denervated heart rate pharmacologically (5).

To date no systematic study of the mechanism of the decline in VCA activity has been performed. Two hypotheses may be proposed to account for the decline: a decrease in response of the peripheral neuroeffector system, and a decrease in the vagal tone. The present study was undertaken to examine these hypotheses in neuroleptanesthetized dogs by comparing the change in heart rate response to electric vagal nerve stimulation with the change in VCA activity during a well defined period of time.

## Materials and Methods

Experiments were conducted on 19 mongrel dogs of both sexes (16-38 kg) in neuroleptanesthesia. Anesthesia was induced by a combination anesthetic of fentanyl (0.1 mg/kg, i.m.) and fluanisone (5 mg/kg, i.m.) (Hypnorm, Janssen); analgesia was maintained by fentanyl (0.01 mg/kg/h, i.v.) (Fentanyl-Janssen, Janssen). Spontaneous ventilation was suppressed by means of alcuronium chloride (3 mg and 0.6 mg/h, i.v.) (Alloferin,

Roche). After endotracheal intubation the dogs were ventilated with a mixture of $N_2O$ and $O_2$ in a ratio of 2:1, using intermittent positive pressure (Pulmomat 19K1). Local anesthesia with lidocaine (Xylocaine spray 10%, Astra) was applied to the areas of the skin that were cut. Minute ventilation was adjusted to maintain end-tidal $pCO_2$ (Capnograph) within 4.0-4.7 kPa. A femoral artery was catheterized using the Seldinger technique (catheter FG 06, Vygon) for taking blood samples for the estimation of $pO_2$, $pCO_2$, pH (Radiometer ABL 2) and oxygen saturation (Radiometer OSM 2). If necessary, sodium bicarbonate (500 mmol/l) was given intravenously to maintain the blood gas parameters within the normal range for unanesthetized dogs (30).

Systemic arterial blood pressure (Statham P23Db) and a lead II electrocardiogram (stainless steel needle electrodes) were recorded on a chart recorder (Mingograph, Elema). HR was determined with a tachometer (ECG module, Philips V 692) and continuously recorded on a chart recorder (Kipp BD 101). The body temperature, determined with an intramuscular thermocouple (Ellab TE3), was maintained in the range of 37.5-38.5 °C by means of a thermostatically controlled heating pad. Two intravenous catheters (Vasocan Braunule OD 1 mm, Braun Melsungen) were used for drug administration and fluid infusion.

The criterion for maintenance of an adequate depth of anesthesia was the absence of responses in HR and arterial blood pressure to noxious stimuli before blocking the autonomic neurotransmission. On completion of the experiments the anesthetized animals were killed with an overdose of KCl solution (1.4 mmol/l). The study protocol was approved by the appropriate Animal Care and Use Committee (Groningen University, FDC 65-0186).

*Experimental protocol*

Twelve dogs were used to examine whether VCA activity is reproducible, in particular with respect to its maximum and the time course of its spontaneous decline. Each animal underwent two types of experiment, separated by at least a 14-day interval. In the first one, the HR responses to successive $\beta$-adrenergic, muscarinic and ganglionic nicotinic receptor blockade was examined (exp. 1). To block the sympathetic cardiac accelerator influence the $\beta$-adrenergic receptors were blocked 15 min after the instrumentation period. HR was allowed to stabilize for 30 min, after which the vagal cardiac decelerator influence was blocked by the administration of a muscarinic receptor antagonist. As a result, HR increased, reaching a maximum level within 10 min after the muscarinic receptor blockade. The subsequent decrease in tachycardia was observed for 170 min. In order to block the vagal cardiac accelerator influence, a ganglionic nicotinic

receptor antagonist was injected subsequently, and the minimum HR was determined. The second type of experiment was similar to the first one except that vagotomy was substituted for the nicotinic receptor blockade (exp. 2).

Two other experiments were performed to assess whether in β-adrenergic and muscarinic receptor blocked dogs the cardiac responsiveness to electric vagal nerve stimulation changes with time. The response in HR to vagal nerve stimulation was determined at two well defined points of time. In a first group of dogs ($n$=7) vagotomy was performed 10 min after the muscarinic receptor blockade. After a subsequent stabilization period vagal nerve stimulation was carried out and the resulting maximum HR determined: 0-min scenario (exp. 3) (Fig. 1*A*). In a second group of dogs ($n$=7) vagotomy was performed 180 min after the muscarinic receptor blockade. Because of the duration of the interval between the maximum HR after muscarinic receptor blockade and the vagotomy this scenario was designated as 170-min scenario (exp. 4) (Fig. 1*B*). The dogs from the second group, which were also used in exp. 1 and 2, were matched for the characteristics of VCA activity with the dogs of the first group.

*Nerve stimulation procedure*

After exposure in the neck, each vagosympathetic trunk was dissected free from connective tissue and transected approximately 5 cm below the nodose ganglion. The distal nerve endings were bathed in paraffin oil to avoid drying-up. Direct stimulation was carried out with two epoxy-coated circular Ag-electrodes (ID 2-3 mm), positioned around the nerve 10 mm apart. Continuous stimulation at a frequency of 20 Hz and a pulse width of 1 ms was provided by a double pulse stimulator (SRI 50-4993) and delivered to the nerve via a stimulus isolation unit (Neurolog NL 800). The vagus nerves were stimulated at supramaximal intensity and a duration of 4 min.

*Drugs*

The β-adrenergic receptors were blocked with timolol (0.2 mg/kg and 0.2 mg/kg/h) (Sigma). Methylatropine (0.5 mg/kg and 0.5 mg/kg/h) (Sigma) was used as a muscarinic receptor antagonist. Ganglionic nicotinic receptor blockade was achieved by hexamethonium (20 mg/kg) (Fluka). All drugs were dissolved in an NaCl solution (154 mmol/l) and administered intravenously.

*Data analysis*

"Inherent VCA" denotes the vagal cardiac acceleration which becomes manifest after muscarinic receptor blockade in the β-adrenoceptor blocked dog. The maximum

"inherent VCA" is the difference between the maximum HR after muscarinic receptor blockade and HR after nicotinic receptor blockade (exp. 1) or vagotomy (exp. 2, 3 and 4). The "inherent VCA" 180 min after muscarinic receptor blockade indicates the difference between HR 180 min after muscarinic receptor blockade and HR after nicotinic receptor blockade (exp. 1) or vagotomy (exp. 2 and 4). "Stimulation VCA" denotes HR response to electric vagal nerve stimulation. The maximum "stimulation VCA" is the difference of the maximum HR after stimulation and HR after vagotomy (exp. 3 and 4).

From exp. 4 the rate of decline of "inherent VCA" was calculated as the difference between the maximum "inherent VCA" and the "inherent VCA" 180 min after muscarinic receptor blockade, divided by the maximum "inherent VCA". From the experiments 3 and 4 the rate of decline of "stimulation VCA" was calculated as the difference in the ratio of maximum "stimulation VCA" and maximum "inherent VCA", between each dog of exp. 3 and its matched counterpart of exp. 4. Values are reported as means $\pm$ S.E.M. Matched comparisons were analyzed using the paired Student's $t$ test. A $P$ value $< 0.05$ was considered significant.

**Results**

As the result of $\beta$-adrenoceptor blockade, HR in exp. 1 and exp. 2 decreased to the same extent (Table 1). The subsequent muscarinic receptor blockade led to an increase from 53 to 208 beats/min (exp. 1) and 56 to 192 beats/min (exp. 2). After attainment of the maximum level within 10 min after the administration of methylatropine, HR spontaneously declined throughout the following 170-min observation period to 154 beats/min (exp. 1) and 140 beats/min (exp. 2). The maximum HR after muscarinic receptor blockade in exp. 2 was significantly lower than in experiment 1, and HR after vagotomy (exp. 2) was significantly lower than after nicotinic receptor blockade (exp. 1). The maximum inherent VCA in the two types of experiment was almost the same: $68 \pm 10$ and $64 \pm 11$ beats/min. Each dog appeared to have its own reproducible maximum inherent VCA. Between dogs the maximum inherent VCA varied considerably, ranging from 15 to 120 beats/min. There were no significant differences in the rate of the decline in VCA activity between the two experiments for any of the chosen time variables (Table 1).

Table 1 Heart rate (beats/min) following β-adrenergic and muscarinic receptor blockade and following subsequent nicotinic receptor blockade (exp. 1) or vagotomy (exp. 2) in one group of dogs.

| | Exp. 1 | Exp. 2 | |
|---|---|---|---|
| basal | 101±12 | 115±15 | |
| β-adrenoceptor block | 53±3 | 56±3 | |
| maximum after muscarinic block | 208±10 | 192±12 | * |
| 180 min after muscarinic block | 154±7 | 140±7 | * |
| nicotinic block | 140±6 | | |
| vagotomy | | 128±6 | ** |
| maximum inherent VCA (beats/min) | 68±10 | 64±11 | |
| inherent VCA 180 min after muscarinic block | 14±2 | 12±3 | |
| decline in inherent VCA (min) | | | |
| t25% | 23±7 | 21±4 | |
| t50% | 42±8 | 51±12 | |
| t75% | 127±18 | 113±20 | |

Values are means ± S.E.M., $n=12$. Asterisk (*) denotes significant difference between exp. 1 and 2 ($P < 0.05$). Asterisk (**) denotes significant difference *versus* the HR after nicotinic receptor blockade in exp. 1. The maximum inherent VCA is the maximum vagal cardiac acceleration, determined by subtracting HR after nicotinic receptor blockade (exp. 1) or vagotomy (exp. 2) from maximum HR after muscarinic receptor blockade. The inherent VCA 180 min after muscarinic receptor blockade is determined by subtracting HR after nicotinic receptor blockade (exp. 1) or vagotomy (exp. 2) from HR 180 min after muscarinic receptor blockade. The parameter ty% is the value of t at which the decline in inherent VCA is y% of the maximum inherent VCA. The ty% data in 4 dogs which exhibited a maximum inherent VCA of less than 25 beats/min, could not be satisfactory determined and were excluded from the results.

The left section of panel *B* of Fig. 1 shows the time course of HR during a 170-min observation period. After the administration of timolol, HR dropped to 67 beats/min. The response to the subsequent muscarinic receptor blockade is characterized by three distinct phases. In the first phase the record exhibits a large and steep slope, lasting about 30 s. The second phase, characteristically separated from the first one by a small notch, shows a slow further increase in HR to a maximum of 232 beats/min. The third phase shows a quasi-exponential decrease in HR. After vagotomy and a 50-min stabilization period, HR reached a value of 128 beats/min, considered to be the denervated HR. The maximum inherent VCA was calculated as 232-128=104 beats/min. During the third phase the inherent VCA declined to 50 and 25% maximum levels after 67 and 97 min, respectively. The difference of 4 beats/min in HR before and after the vagotomy represents the inherent VCA prior to vagotomy.

In exp. 3 and exp. 4 the HR response to electric vagal nerve stimulation was assessed at two different points of time, vagotomy being performed either 0 or 170 min

after the maximum HR following the muscarinic receptor blockade in the β-adrenoceptor blocked dog (Table 2). In the 0-min scenario, HR increased from 134±9 to 184±12 beats/min, the maximum stimulation VCA thus being 184-134=50 beats/min. In the 170-min scenario HR increased from 136±5 to 160±9 beats/min, corresponding with a maximum stimulation VCA of 24±8 beats/min.

When expressed as percentage of the maximum inherent VCA, the maximum stimulation VCA in the 0-min and 170-min scenario was 116±7% and 41±8%, respectively. The maximum stimulation VCA and the ratio maximum stimulation VCA / maximum inherent VCA differed significantly between exp. 3 and exp. 4. No statistically significant difference was found between the rate of decline of inherent VCA and the rate of decline of stimulation VCA. The rate of decline of inherent VCA, calculated from exp. 4, amounted to 77±6% (=(49-11)/49 beats/min); the rate of decline of stimulation VCA, calculated from exp. 3 and exp. 4 was 75±10% (116%-41%).

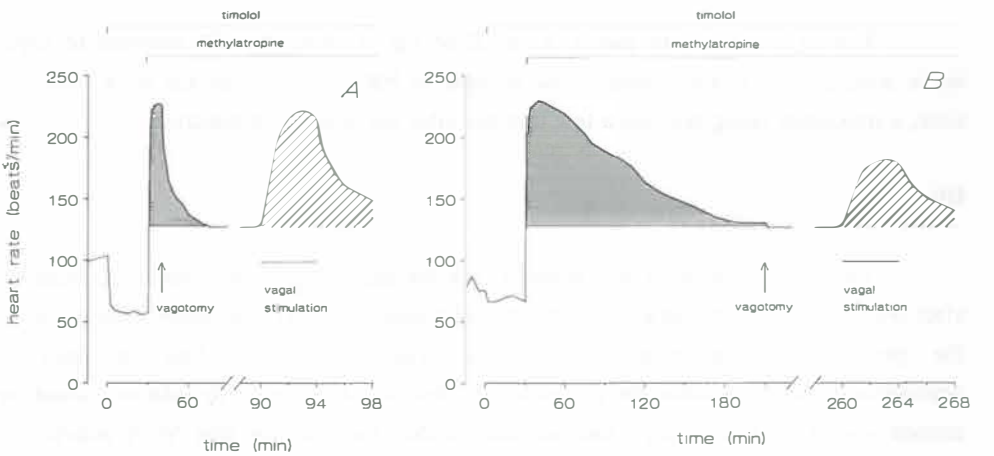



Fig. 1 Effect of timing of vagotomy on the heart rate (beats/min) response to vagal nerve stimulation in two β-adrenoceptor and muscarinic receptor blocked dogs in neuroleptanesthesia. The β-adrenoceptors were blocked with timolol, muscarinic receptors with methylatropine. Each panel compares the maximum HR response to vagal nerve stimulation (20 Hz, 1 ms, supramaximal) (hatched area) with the maximum inherent VCA (shaded area). Note that the HR response to vagal nerve stimulation is dependent on the time interval between muscarinic receptor blockade and vagal nerve stimulation; a considerably larger HR increase is observed when vagotomy was performed 10 min after muscarinic receptor blockade (panel *A*; exp. 3) than when vagotomy was performed 180 min after muscarinic blockade (panel *B*; exp. 4). Time scale is stretched after the onset of stimulation to highlight the time course of the increase in HR.

Table 2 Maximum heart rate (beats/min) response to vagal stimulation according to a 0-min scenario (exp. 3) and a 170-min scenario (exp. 4) in two groups of $\beta$-adrenoceptor and muscarinic receptor blocked, vagotomized dogs matched for maximum inherent VCA.

| | Exp. 3 | Exp. 4 | |
|---|---|---|---|
| interval between maximum after muscarinic block and vagotomy (min) | 0 | 170 | |
| maximum inherent VCA | 45±11 | 49±12 | |
| inherent VCA 180 min after muscarinic block | | 11±2 | |
| HR after $\beta$-adrenergic and muscarinic block and vagotomy | 134±9 | 136±5 | |
| maximum HR during vagal stimulation | 184±12 | 160±9 | * |
| maximum stimulation VCA | 50±9 | 24±8 | * |
| stimulation VCA / max inherent VCA (%) | 116±7 | 41±8 | * |

Values are means ± S.E.M., $n$=7. Asterisk (*) denotes significant difference between exp. 3 and 4 ($P < 0.05$). Maximum inherent VCA is determined by subtracting HR after vagotomy from maximum HR after muscarinic receptor blockade. The inherent VCA 180 min after muscarinic receptor blockade is determined by subtracting HR after vagotomy from HR 180 min after muscarinic receptor blockade.

The right sections of panels *A* and *B* of Fig. 1 show the HR response to vagal nerve stimulation. In both scenarios the increase in HR was characterized by a slow rise time, a maximum being reached a few minutes after the onset of stimulation.

## Discussion

The major finding of this study is that the decline in vagal cardiac acceleration after $\beta$-adrenoceptor and muscarinic receptor blockade is due to a decrease in response of the peripheral neuroeffector system. Our experiments were done in dogs in neuroleptanesthesia, because the procedure of electric vagal nerve stimulation cannot be carried out in conscious dogs and previous studies have shown that VCA activity, if present at all, is considerably reduced in general anesthesia with pentobarbital (4, 11), $\alpha$-chloralose (9, 11), and methoxyflurane (4) due to their depressant actions on subcortical functions (4). However, in $\beta$-adrenoceptor blocked dogs that are in neuroleptanesthesia, HR increases to similar high levels after blocking the muscarinic receptors than observed in $\beta$-adrenoceptor blocked conscious dogs (4, 11, 25). Hence, the VCA activity in animals during neuroleptanesthesia and in the conscious state are comparable. Although the analgetic fentanyl, used in neuroleptanesthesia, has been reported to increase the vagal tone (14, 17, 18, 19), it seems to enhance only the vagal decelerator tone, as signified by the low HR following $\beta$-adrenoceptor blockade in the exp. 1 and exp. 2 (Table 1), but

leaves the vagal cardiac accelerator tone unaltered, as indicated by the similar VCA activities in the neuroleptanesthetized and conscious dogs (4, 11, 25).

The aim of exp. 1 and 2 was to examine the reproducibility of the VCA activity. The absence of a significant difference in the maximum inherent VCA (Table 1) validates its use as reference value in the stimulation exp. 3 and 4. In fact, each dog appeared to have its own characteristic maximum inherent VCA, reproducible within narrow limits. Between dogs, however, the maximum inherent VCA varied considerably. In exp. 1 and 2 the inherent VCA declined to 50 and 25% maximum levels after about 45 and 120 min, respectively. In conscious dogs, however, VCA activity declined more rapidly. Rigel et al. (25) reported that the inherent VCA (excess tachycardia in their terminology) declined to 50 and 20% maximum levels after 30 and 70 min, respectively. These authors did not speculate about the mechanism underlying the decline.

Evidence in support of the decline in VCA activity being due to a decrease in neuroeffector response is provided by the results of exp. 3 and 4. The maximum increase in HR in response to electric vagal nerve stimulation, *i.e.* maximum stimulation VCA, appeared to be lower if the interval between vagotomy and muscarinic receptor blockade was longer (Fig. 1). When expressed as percentage of the maximum inherent VCA, the maximum stimulation VCA according to the 0-min and 170-min scenario was 116 and 41%, respectively (Table 2). This implies that the peripheral neuroeffector responsiveness declines by 75% over the time period of 170 min. The inherent VCA was at its maximum about 10 min after the muscarinic receptor blockade and had declined by 77% over the time period of 170 min. Therefore, the observed decline in peripheral neuroeffector responsiveness is sufficient to account for the observed decline of inherent VCA.

Although we have not conducted a systematic investigation of the relationship between the frequency of vagal nerve stimulation and change in HR, the positive chronotropic effect of vagal nerve stimulation was found to be most pronounced at 20 Hz (Schuil HA and Patberg WR, unpublished). Similar results have been recently published by Henning (15) and Hill et al. (16). Comparison of the maximum stimulation VCA and maximum inherent VCA in the 0-min scenario (Fig. 1*A*) proves that it was possible to simulate the natural activity of the vagal cardiac accelerator system.

The explanation for the decrease in the peripheral neuroeffector response is probably related to the release of neurotransmitter from the vagus nerves, although a desensitization of the pacemaker receptors cannot be ruled out. In view of the fact that the frequency of vagal nerve stimulation (20 Hz) in our study is comparable with the frequency of nerve stimulation that in various tissues results in a maximum release of neuropeptides (1, 10, 23), it is tempting to speculate that a neuropeptide is the responsible

transmitter in the VCA pathway. It this connection, it has been suggested that vasoactive intestinal polypeptide (VIP) produces vagal cardiac acceleration, because VIP-immunoreactivity has been found in cholinergic nerve fibers and it is suggested that VIP is co-localized with acetylcholine within the same nerve terminals in cardiac vagal nerves (31). Furthermore, intracoronary injection of VIP in anesthetized (26) and conscious dogs (27) has been shown to increase HR, despite the presence of $\beta$-adrenoceptor and muscarinic receptor antagonists. One mechanism that leads to the decrease of neuroeffector responsiveness is the exhaustion of peptide stores at the nerve terminals. It has been demonstrated that by the administration of methylatropine these stores are depleted as a consequence of the loss of muscarinic autoreceptor-mediated inhibition on peptide (e.g. VIP) release (1). In this situation the replenishment of neuropeptides by axonal transport from the cell body may be insufficient and consequently result in a spontaneous decline in inherent VCA.

An additional factor that might contribute to the decrease in neuroeffector responsiveness, is that the release of the neurotransmitter which produces VCA is influenced by transneuronal modulation (6, 24). In this connection, two transmitters released by sympathetic nerve fibers are worthwhile to be considered. Noradrenaline is known to attenuate vagal activity, though its effect is short-lasting (22). The peptide NPY has a similar, but long-lasting, effect (20, 22). Evidence for a modulation of the VCA activity by the sympathetic nervous division can be inferred from the results of Donald et al. (11), who found the greatest cardioaccelerator response to vagal stimulation in reserpinized dogs. Reserpine causes depletion of catecholamines- and NPY-stores in sympathetic nerve terminals (21) and thereby the loss of this transneuronal modulation. However, if present, the sympathetic mediated transneuronal modulation will only in part be responsible for the decline in VCA activity, because VCA activity decreased also in sympathectomized and in reserpinized dogs (3, 4, 11, 13).

The present study was not designed to determine the denervated HR, which is strictly defined as the frequency at which the heart beats when the cardiac neural input is removed, in our experiments an approximation of the denervated HR was obtained by effector blockade in combination with ganglionic nicotinic receptor blockade or vagotomy. The resulting heart rates compared reasonably well with published data obtained under experimental conditions resembling those in the present study (5, 12). However, HR after nicotinic receptor blockade ($140\pm6$ beats/min) was significantly higher than after vagotomy ($128\pm6$ beats/min) (Table I). This seemed not necessarily to be due to the employed experimental procedure since already before nicotinic receptors were blocked and vagotomy was performed a significant difference in HR was found. This difference

might be a reflection of changes in the concentration of local autacoids, influencing HR directly, or might be related to differences in hemodynamic parameters, such as mean circulatory filling pressure or coronary perfusion pressure.

In summary, a decrease in peripheral neuroeffector responsiveness is demonstrated to underlie the spontaneous decline in inherent VCA. The decline in inherent VCA appeared to be as marked as the simultaneous decrease in stimulation-induced VCA activity. No arguments are found to assume that a decrease in vagal accelerator tone accounts for the decline in inherent VCA. Our findings form experimental evidence for the suggestion put forward by Chassaing et al. (8) that the decline in VCA activity is due to peripheral factors only.

## References


1. Bartfai T, Iverfeldt K, Fisone G (1988) Regulation of the release of coexisting neurotransmitters. *Annu Rev Pharmacol Toxicol* 28:285-310
2. Brouha L, Cannon WB, Dill DB (1936) The heart rate of the sympathectomized dog in rest and exercise. *J Physiol* 87:345-359
3. Brouha L, Nowak SJG (1939) The role of the vagus in the cardio-accelerator action of atropine in sympathectomized dogs. *J Physiol* 95:439-453
4. Brunsting JR, Bennekers JH, Schuil HA, Zijlstra WG (1979) Incomplete cardiac vagal blockade with atropine in the anesthetized dog. *Pflügers Arch* 381:293-295
5. Brunsting JR, Schuil HA, Zijlstra WG (1983) Intrinsic heart rate in the dog determined by pharmacologic denervation. *Am J Physiol* 245:H592-H597
6. Burnstock G (1986) The changing face of autonomic neurotransmission. *Acta Physiol Scand* 126: 67-91
7. Chassaing C, Duchêne-Marullaz P, Moins N, Combre A (1976) Effets paradoxaux de propranolol et d'atropine sur la fréquence cardiaque du chien non narcosé. *J Pharmacol* 5:609-618
8. Chassaing C, Godeneche D, Boucher M, Duchêne-Marullaz P (1979) A comparison of changes in atropine-induced tachycardia and atropine concentration in conscious dogs. *Eur J Pharmacol* 58: 433-441
9. Chassaing C, Boucher M, Breysse C, Duchêne-Marullaz P (1992) Contribution of vagal blockade to the tachycardia induced by the antimuscarinic agent atropine and pirenzepine. *J Auton Pharmacol* 12:359-368
10. Corr L (1992) Neuropeptides and the conduction system of the heart. *Int J Cardiol* 35:1-12
11. Donald DE, Samueloff SL, Ferguson D (1967) Mechanisms of tachycardia caused by atropine in conscious dogs. *Am J Physiol* 212:901-910
12. Evans JM, Randall DC, Funk JN, Knapp CF (1990) Influence of cardiac innervation on intrinsic heart rate in dogs. *Am J Physiol* 258:H1132-H1137
13. Freeman NE, Page IH (1937) Hypertension produced by constriction of the renal artery in sympathectomized dogs. *Am Heart J* 14:405-414
14. Freye E, Arndt JO (1979) Perfusion of the fourth cerebral ventricle with fentanyl induces naloxone-reversible bradycardia, hypotension, and EEG synchronisation in conscious dogs.

*Naunyn-Schmiedeberg's Arch Pharmacol* 307:123-128

15. Henning RJ (1992) Vagal stimulation during muscarinic and β-adrenergic blockade increases atrial contractility and heart rate. *J Auton Nerv Syst* 40:121-130
16. Hill MRS, Wallick DW, Martin PJ, Levy MN (1990) Excess tachycardia: an anesthetized preparation and its stimulation frequency response. *Proc Annu Int Conf IEEE Eng Med Biol Soc* 12:757-758
17. Inoue K, Samodelov LF, Arndt JO (1980) Fentanyl activates a particular population of vagal efferents which are cardioinhibitory. *Naunyn-Schmiedeberg's Arch Pharmacol* 312:57-61
18. Laubie M, Schmitt H, Drouillat M (1977) Central sites and mechanisms of the hypotensive and bradycardic effects of the narcotic analgesic agent fentanyl. *Naunyn-Schmiedeberg's Arch Pharmacol* 296:255-261
19. Laubie M, Schmitt H (1980) Action of the morphinomimetic agent, fentanyl, on the nucleus tractus solitarii and the nucleus ambiguus cardiovascular neurons. *Eur J Pharmacol* 67:403-412
20. Moriarty M, Gibbins IL, Potter EK, McCloskey DI (1992) Comparison of the inhibitory roles of neuropeptide Y and galanin on cardiac vagal action in the dog. *Neurosci Lett* 136:275-279
21. Pernow J, Thorén P, Millberg B-I, Lundberg JM (1988) Renal sympathetic nerve activation in relation to reserpine-induced depletion of neuropeptide Y in the kidney of the rat. *Acta Physiol Scand* 134: 53-59
22. Potter EK (1985) Prolonged non-adrenergic inhibition of cardiac vagal action following sympathetic stimulation: neuromodulation by neuropeptide Y? *Neurosci Lett* 54:117-121
23. Potter EK (1988) Neuropeptide Y as an autonomic neurotransmitter. *Pharmacol Ther* 37:251-273
24. Rand MJ, Majewski H, Wong-Dusting H, Story DF, Loiacono RE, Ziogas J (1987) Modulation of neuroeffector transmission. *J Cardiovasc Pharmacol* 10:S33-S44
26. Rigel DF (1988) Effects of neuropeptides on heart rate in dogs: comparison of VIP, PHI, NPY, CGRP, and NT. *Am J Physiol* 255:H311-H317
25. Rigel DF, Lipson D, Katona PG (1984) Excess tachycardia: heart rate after antimuscarinic agents in conscious dogs, *Am J Physiol* 246:H168-H173
27. Roossien A, Brunsting JR, Zaagsma J, Zijlstra WG (1992) Vasoactive intestinal polypeptide: a probable mediator of vagal cardioacceleration (VCA) in dogs. *Pflügers Arch* 420:R88
28. Schuil HA, Brunsting JR, van der Molen H, Zijlstra WG (1981) Cardioacceleratory effect of muscarinic blocking agents in the dog. *Eur J Pharmacol* 69:229-233
29. Schuil HA, Brunsting JR, Zijlstra WG (1983) Some aspects of the nature of the non-muscarinic, non-adrenergic cardioacceleratory pathway in the dog. *Arch Int Physiol Biochim* 91:P6-P7
30. Zweens J, Frankena H, Kampen EJ van, Rispens P, Zijlstra WG (1977) Ionic composition of arterial and mixed venous plasma in the unanesthetized dog. *Am J Physiol* 233:F412-F415
31. Weihe E, Reinecke M, Forssmann WG (1984) Distribution of vasoactive intestinal polypeptide-like immunoreactivity in the mammalian heart. *Cell Tissue Res* 236:527-540

# 3

## INFLUENCE OF NEUROLEPTANESTHESIA ON VAGAL CARDIAC ACCELERATION


**Abstract**

The vagal cardiac acceleration (VCA), which becomes manifest after blocking the $\beta$-adrenoceptors (timolol 0.2 mg/kg and 0.2 mg/kg/h, i.v.) and muscarinic receptors (methylatropine 0.5 mg/kg and 0.5 mg/kg/h, i.v.) and which is abolished by nicotinic receptor blockade (hexamethonium 20 mg/kg, i.v.), was investigated in dogs in the conscious and neuroleptanesthetized state. Neuroleptanesthesia was induced by a combination anesthetic of fentanyl (0.1 mg/kg, i.m.) and fluanisone (5 mg/kg, i.m.). Furthermore, the influence of preceding $\alpha_1$-adrenergic receptor blockade (doxazosin 0.1 mg/kg, i.v.) on VCA was examined. Maximum VCA was defined as the heart rate (HR) after nicotinic receptor blockade subtracted from the maximum HR after muscarinic receptor blockade.

In $\beta$-adrenoceptor blocked dogs (protocol I), HR reached a comparable maximum level following muscarinic receptor blockade in the anesthetized ($195\pm9$ beats/min) and conscious ($202\pm9$ beats/min) state. In the following 2h HR declined to a comparable level of 150 beats/min in both conditions. After nicotinic receptor blockade, HR decreased $25\pm3$ beats/min and stabilized at a level of $123\pm3$ beats/min in conscious dogs, whereas during anesthesia, HR decreased significantly less ($17\pm3$ beats/min) and stabilized at a significantly higher level of $135\pm5$ beats/min. As a consequence, maximum VCA was greater in conscious ($79\pm8$ beats/min) than in anesthetized dogs ($60\pm8$ beats/min). In $\alpha_1$- and $\beta$-adrenoceptor blocked dogs (protocol II), the maximum HR after muscarinic receptor blockade reached a lower level in the conscious ($180\pm15$ beats/min) than in the anesthetized dogs ($195\pm15$ beats/min). After subsequent nicotinic receptor blockade, HR decreased $20\pm2$ beats/min and stabilized at $119\pm6$ beats/min in the conscious state, and in anesthesia HR decreased $18\pm5$ beats/min and stabilized at a significantly higher level ($137\pm5$ beats/min). Consequently, maximum VCA amounted to $61\pm12$ beats/min in the conscious state and $58\pm13$ beats/min in anesthesia.

Comparison of the two protocols showed that in the conscious state maximum

VCA was significantly decreased by $\alpha_1$-adrenoceptor blockade, whereas in anesthesia maximum VCA was unaltered. The discrepancies in HR responses to muscarinic and nicotinic receptor blockade between the conscious and anesthetized state are probably related to the distinct responses in mean arterial blood pressure (MAP) to these interventions. Following muscarinic blockade a maximum increase in MAP of $61\pm5$ mmHg was observed in $\beta$-adrenoceptor blocked dogs in anesthesia, while in the conscious state MAP increased $25\pm4$ mmHg (protocol I). The increase in MAP was prolonged during anesthesia and transient in the conscious state. In $\alpha_1$- and $\beta$-adrenoceptor blocked dogs (protocol II), the increase in MAP following muscarinic receptor blockade in anesthesia was comparable to that observed in protocol I ($54\pm6$ mmHg), while in the conscious state MAP increased $18\pm5$ mmHg which was, unlike in protocol I, followed by a transient decrease before returning to its previous level. Following nicotinic receptor blockade, MAP decreased to significantly lower levels in the conscious state ($64\pm6$ and $56\pm4$ mmHg in protocol I and II, respectively) than in the anesthetized state ($107\pm6$ and $102\pm6$ mmHg). Furthermore, it was recognized that, unlike conscious dogs, anesthetized dogs did not respond to $\alpha_1$-adrenoceptor blockade with alterations in MAP and HR. Although neuroleptanesthesia has mostly been considered the anesthesia of choice in investigating VCA, the present study shows that even in this kind of anesthesia the vagal accelerator control of the heart rate is affected.

## Introduction

Heart rate is mainly controlled by the autonomic nervous system. It is commonly acknowledged that the autonomic nervous system exerts an accelerator influence through sympathetic nerve fibers and a decelerator influence through vagal nerve fibers. Brouha et al. (3) and Freeman and Page (13), however, recognized that the vagus also mediates a cardiac accelerator influence. After blocking muscarinic receptors in sympathectomized conscious dogs, these authors observed a cardiac acceleration, which was not observed when the vagal nerves had been transected. It was established that the vagal cardiac acceleration (VCA) is not produced by catecholamine activation of $\beta$-adrenergic receptors, because VCA was also present in muscarinic receptor blocked dogs which were either treated with a $\beta$-adrenoceptor antagonist (4, 9, 27) or pretreated with reserpine (9), which causes depletion of catecholamine stores in the sympathetic nerve terminals.

Various authors have reported that VCA is considerably reduced or even absent during anesthesia with pentobarbital (3, 5, 9), $\alpha$-chloralose (7, 9) and methoxyflurane (4), probably because of their central depressant and vagolytic actions (3, 5, 9). In contrast,

neuroleptanesthesia, which in the dog is induced by means of a combination of the neuroleptic fluanisone and analgetic fentanyl, has been claimed the anesthesia of choice in investigating VCA, because in $\alpha$- and $\beta$-adrenoceptor blocked neuroleptanesthetized dogs the heart rate increased to a similar high maximum level following muscarinic receptor blockade as was found in $\beta$-adrenoceptor blocked conscious dogs. Moreover, in neuroleptanesthetized dogs (4, 31) VCA has a transient nature similar to that described in the conscious state (3, 9, 13).

With the use of neuroleptanesthesia, Brunsting et al. (5, 6) and Schuil et al. (30, 31) were able to confirm the vagal origin of the acceleration by showing that VCA disappeared by transecting the vagus nerves. Furthermore, they established that VCA disappeared following the administration of a nicotinic receptor antagonist (4, 5, 30, 31). As a consequence, it was possible to quantify VCA (4) and to determine the denervated heart rate in intact dogs pharmacologically (5). However, the relationship between VCA and arterial blood pressure, as established in conscious dogs by Rigel et al. (27), could not be demonstrated in $\alpha$- and $\beta$-adrenergic and muscarinic receptor blocked dogs which were in neuroleptanesthesia (31).

The present study was designed to compare VCA in neuroleptanesthesia with VCA in the conscious state in $\beta$-adrenoceptor blocked dogs, either with or without $\alpha_1$-adrenoceptor blockade.

## Methods

*Surgical preparation*

Fifteen mongrel dogs of either sex (18-36 kg) were used in this study. Prior to experimentation, dogs were instrumented using aseptic surgical techniques. Surgery was carried out under neuroleptanesthesia. Indwelling catheters (Tygon microbore S-54-HL, ID 0.5 mm, OD 1.5 mm) were inserted into the right omocervical vein and artery and the tips advanced to the superior caval vein (drug infusion) and descending aorta (blood pressure measurement). The catheters were tunnelled subcutaneously and secured to the skin in the midscapular region. Catheters were kept patent by daily flushing with heparinized saline (2000 U/ml). The dogs wore jackets to protect the catheters from being chewed on. At least 2 weeks passed between surgery and the start of the experiments. The study protocol was approved by the appropriate Animal Care and Use Committee (Groningen University, FDC 65-0186).

*Protocol*

There were two experimental protocols. In each protocol two types of experiment, separated by at least a 14-day interval, were performed: one in neuroleptanesthesia and one in the conscious state. In the first protocol (protocol I, $n=15$) the responses in heart rate (HR) and mean arterial blood pressure (MAP) to successive $\beta$-adrenergic, muscarinic and nicotinic receptor blockade were examined. The $\beta$-adrenergic and muscarinic receptors were blocked 15 and 45 min after the start of the experiment, respectively. After a 2-h observation period, ganglionic nicotinic receptor blockade was carried out and after a stabilization period of 35 min HR and MAP were determined. The second protocol (protocol II, $n=7$) differed from protocol I in an additional $\alpha_1$-adrenergic receptor blockade established 10 min before the $\beta$-adrenergic blockade.

*Conditioning*

Neuroleptanesthesia was induced by a combination anesthetic of fentanyl (0.1 mg/kg, i.m.) and fluanisone (5 mg/kg, i.m.) (Hypnorm, Janssen); analgesia was maintained by fentanyl (0.01 mg/kg/h, i.v.) (Fentanyl-Janssen, Janssen). Spontaneous ventilation was suppressed by means of vecuronium bromide (4 mg and 1 mg/h, i.v.) (Norcuron, Organon Teknika). After endotracheal intubation the dogs were ventilated with a mixture of $N_2O$ and $O_2$ in a ratio of 2:1, using intermittent positive pressure (Pulmomat 19K1). Minute ventilation was adjusted to maintain end-tidal $pCO_2$ (Capnograph) within 4.0-4.7 kPa. Blood samples were taken for the estimation of $pO_2$, $pCO_2$ and pH (Radiometer ABL2) and oxygen saturation (Radiometer OSM2). If necessary, sodium bicarbonate (500 mmol/l) was given intravenously to maintain the blood gas parameters within the normal range for unanesthetized dogs. Body temperature, determined with an intramuscular thermocouple (Ellab TE3), was maintained within 37.5-38.5 °C by a thermostatically controlled heating pad. Before experiments in the conscious state were performed, the dogs were habituated to the laboratory room and personnel, and trained to stand quietly on a padded table in a modified Pavlovian sling. To minimize external influences that might excite the animals, the room was dimly lit, and the sound level kept as low as possible.

*Measurements and data analysis*

Ag/AgCl electrodes (Quinton) were attached to the shaved paws of the dog to take a lead II ECG. The heart rate was obtained from the ECG by means of a cardiotachometer (ECG module, Philips V 692). Systemic arterial blood pressure was measured through the implanted aortic catheter (Statham P23Db). The signals were

amplified, analog-to-digital converted, and fed into a computer for data processing and display. The data were also registered on chart recorders (Kipp BD 101; Elema Mingograph). "VCA" denotes the vagal cardiac acceleration which becomes manifest after muscarinic receptor blockade in $\beta$-adrenoceptor blocked dogs. "VCA(max)" is defined as the difference between the maximum HR after muscarinic receptor blockade and the HR after nicotinic receptor blockade. "VCA(120)" is the difference between HR 120 min after muscarinic receptor blockade and HR after nicotinic receptor blockade. The maximum change in MAP in response to muscarinic receptor blockade, which typically occurred within 1 min after muscarinic receptor blockade, was denoted $\Delta P_M$ and the maximum change in response to nicotinic receptor blockade $\Delta P_N$. Values reported are means $\pm$ S.E.M. Results were analyzed using Student's paired *t*-test. A $P$ value $< 0.05$ was considered significant.

*Drugs*

The $\alpha_1$-adrenergic receptors were blocked with doxazosin (0.1 mg/kg, i.v.) (Pfizer) and the $\beta$-adrenergic receptors with timolol (0.2 mg/kg and 0.2 mg/kg/h, i.v.) (Sigma). Methylatropine (0.5 mg/kg and 0.5 mg/kg/h, i.v.) (Sigma) was used to block the muscarinic receptors. Ganglionic nicotinic receptor blockade was achieved by hexamethonium (20 mg/kg, i.v.) (Fluka). All drugs were dissolved in an NaCl solution (154 mmol/l).

**Results**

In the experiments of protocol I, HR stabilized at a significantly higher level after $\beta$-adrenergic receptor blockade in the conscious state than during neuroleptanesthesia (Table 1; Fig. 1*A*, *B*). After blocking the vagal decelerator activity, in both types of experiment HR increased to a maximum level of about 200 beats/min, which was typically reached within 10 min, and subsequently decreased to about 150 beats/min in the following 2-h observation period. After additional nicotinic receptor blockade HR dropped by $25 \pm 3$ beats/min in conscious dogs ("VCA(120)"). Following nicotinic receptor blockade in anesthetized dogs HR dropped significantly less ($17 \pm 3$ beats/min). Correspondingly, "VCA(max)" differed significantly in the two types of experiment. In the conscious state, VCA(max) amounted to $79 \pm 8$ beats/min with a range of 12-123 beats/min, while during anesthesia VCA(max) amounted to only $60 \pm 8$ with a range of 5-116 beats/min. In the experiments of protocol II, a decrease in MAP and a reflex tachycardia was observed in the conscious dogs due to the $\alpha_1$-adrenoceptor blockade,

Table 1 Heart rate (beats/min) following subsequent $\beta$-adrenergic, muscarinic and nicotinic receptor blockade according to protocol I and following $\alpha_1$-adrenergic, $\beta$-adrenergic, muscarinic and nicotinic receptor blockade according to protocol II in conscious (Consc) and neuroleptanesthetized (NLA) dogs.

| | Protocol I ($n$=15) | | | Protocol II ($n$=7) | | |
|---|---|---|---|---|---|---|
| | Consc | NLA | | Consc | NLA | |
| basal | 99±6 | 93±7 | | 103±10 | 107±6 | ** |
| $\alpha_1$-adrenergic block | | | | 173±18 | 107±7 | * |
| $\beta$-adrenergic block | 75±5 | 51±4 | * | 86±6 | 56±2 | * |
| maximum after muscarinic block | 202±9 | 195±9 | | 180±15 | ** 195±15 | |
| 120 min after muscarinic block | 148±5 | 152±6 | | 139±8 | 155±9 | * |
| nicotinic block | 123±3 | 135±5 | * | 119±6 | 137±5 | * |
| VCA (max) (beats/min) | 79±8 | 60±8 | * | 61±12 | ** 58±13 | |
| VCA (120) (beats/min) | 25±3 | 17±3 | * | 20±2 | 18±5 | |

Values are means ± S.E.M. Asterisk (*) denotes significant differences compared to the same experiment in the conscious state. Asterisks (**) denotes significant differences compared to the corresponding experiment in protocol I ($P < 0.05$). VCA (max) and VCA (120) signify the maximum vagal cardiac acceleration, determined by subtracting HR after nicotinic receptor blockade from maximum HR after muscarinic receptor blockade and the vagal cardiac acceleration 120 min after muscarinic receptor blockade, determined by subtracting HR after nicotinic receptor blockade from HR 120 min after muscarinic blockade.

whereas during neuroleptanesthesia these reactions were absent (Table 1; Fig. 2*A* & *B*). After subsequent $\beta$-adrenoceptor blockade HR decreased to 86±6 beats/min in conscious dogs and to a significantly lower level of 56±2 beats/min in anesthetized dogs. After muscarinic receptor blockade, HR increased to a lower level in the conscious than in the anesthetized state (180±15 vs. 195± 15 beats/min). Prior to nicotinic receptor blockade HR stabilized at a lower level in the conscious than in the anesthetized state (139±8 vs. 155±9 beats/min). Following nicotinic receptor blockade HR decreased to the same extent, about 20 beats/min, and stabilized at a significantly lower level in the conscious state (119±6 beats/min) compared to the anesthetized state (137±5 beats/min). There were no significant differences in VCA(max) between the two types of experiment.In the conscious state, VCA(max) amounted to 61±12 beats/min with a range of 15-86 beats/min, while during anesthesia VCA(max) amounted to 58±13 with a range of 4-101 beats/min.

In the conscious state the maximum HR after muscarinic receptor blockade and VCA(max) were significantly lower in the presence (protocol II) than in the absence (protocol I) of the $\alpha_1$-adrenoceptor antagonist. Although prior to muscarinic receptor blockade MAP was similar in the two types of experiments of protocol I, the responses to

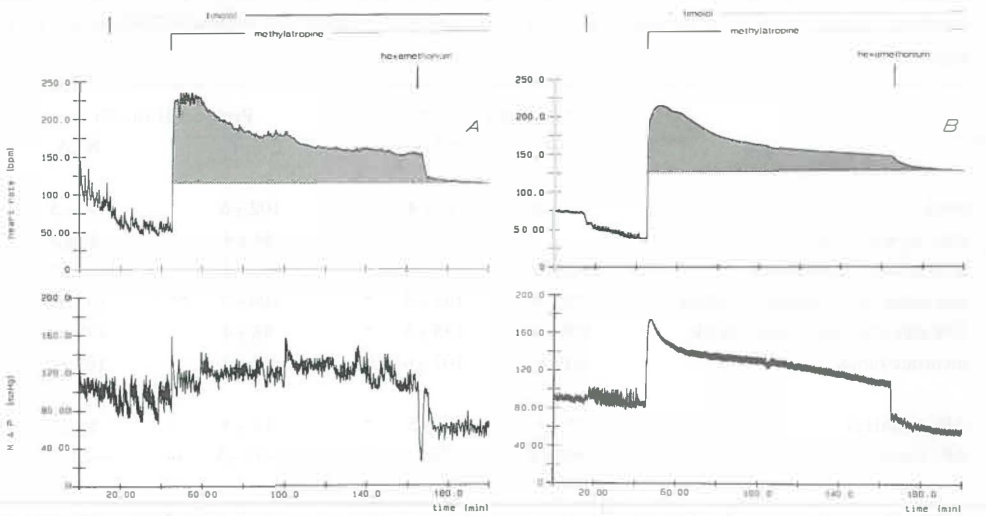



Fig. 1 Heart rate (beats/min) and mean arterial blood pressure (MAP; mmHg) responses to successive β-adrenoceptor, muscarinic and nicotinic receptor blockade (protocol I) in the conscious (panel *A*) and anesthetized (panel *B*) state. The β-adrenoceptors were blocked with timolol, muscarinic receptors with methylatropine and nicotinic receptors with hexamethonium. Vagal cardiac acceleration is represented by the shaded area.

muscarinic and nicotinic receptor blockade differed significantly (Table 2, Fig. 1*A* & *B*). In protocol II the conscious animals showed a brief increase in MAP of 18±5 mmHg following muscarinic receptor blockade and a decrease of 32±5 mmHg following nicotinic receptor blockade and stabilized at a level of 56±4 mmHg (Table 2, Fig. 2*A*). During anesthesia, the increase in MAP following muscarinic receptor blockade was significantly larger (54±6 mmHg) (Fig. 2*B*). Following subsequent nicotinic receptor blockade, MAP decreased by 23±3 mmHg and stabilized at a significantly higher level (102±6 mmHg) than observed in the conscious state. In the anesthetized state the increase in MAP following muscarinic receptor blockade was prolonged, whereas in the conscious state the increase was transient and, unlike in protocol I, followed by a brief decrease before stabilizing at the same level as observed before muscarinic blockade.

Table 2 Mean arterial blood pressure (mmHg) following subsequent β-adrenergic, muscarinic and nicotinic receptor blockade according to protocol I and following $\alpha_1$-adrenergic, β-adrenergic, muscarinic and nicotinic receptor blockade according to protocol II in conscious (Consc) and neuroleptanesthetized (NLA) dogs.

| | Protocol I ($n=15$) | | | Protocol II ($n=7$) | | | |
|---|---|---|---|---|---|---|---|
| | Consc | NLA | | Consc | | NLA | |
| basal | 102±6 | 93±4 | | 102±6 | | 90±5 | |
| after $\alpha_1$-adrenergic block | | | | 84±4 | | 93±5 | |
| after β-adrenergic block | 102±6 | 101±4 | | 86±4 | | 99±5 | |
| maximum after muscarinic block | 128±6 | 162±3 | * | 104±7 | ** [1] | 153±6 | * |
| 120 min after muscarinic block | 109±4 | 135±5 | * | 88±4 | | 125±8 | * |
| nicotinic block | 64±6 | 107±6 | * | 56±4 | | 102±6 | * |
| $\Delta P_M$ (mmHg) | 25±4 | 61±5 | * | 18±5 | | 54±6 | * |
| $\Delta P_N$ (mmHg) | —45±5 | —28±3 | * | —32±5 | | —23±3 | |

Values are means ± S.E.M. Asterisk (*) denote significant differences compared to the same experiment in the conscious state. Asterisks (**) denotes significant difference compared to the corresponding experiment in protocol I ($P<0.05$). $\Delta P_M$ and $\Delta P_N$ signify the maximum responses in MAP to muscarinic and nicotinic receptor blockade, respectively. [1] The transient increase in MAP to 104±7 mmHg was followed by a brief decrease to 74±9 mmHg before reaching the same level as observed before muscarinic receptor blockade.
In the conscious state, MAP increased by 25±4 mmHg following muscarinic receptor blockade and decreased by 45±5 mmHg after nicotinic receptor blockade, whereas in anesthesia MAP increased 61±5 mmHg and decreased 28±3 mmHg, respectively. Furthermore, the increase in MAP in the conscious state was transient, while in anesthesia the increase was prolonged.

## Discussion

Neuroleptanesthesia has been recommended as a suitable anesthesia in studying the vagal cardiac accelerator system, because the magnitude and the transient nature of VCA in this kind of anesthesia are very similar to that described in dogs in the conscious state. That the magnitude of VCA is comparable in these conditions was inferred from the finding that the HR increased to comparable maximum levels after blocking the adrenergic and muscarinic receptors (4). In the present study in β-adrenoceptor blocked (protocol I) and in $\alpha_1$- and β-adrenoceptor blocked dogs (protocol II), however, it was established that VCA appeared to be affected by neuroleptanesthesia. In β-adrenoceptor and muscarinic receptor blocked dogs in anesthesia, the HR reached a similar maximum level as found in the conscious state.

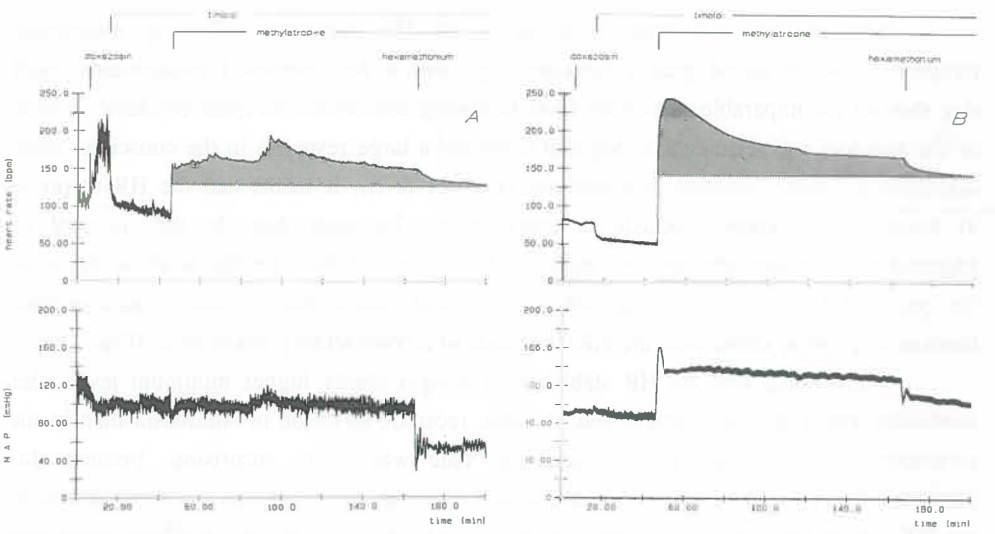



Fig. 2 Heart rate (beats/min) and mean arterial blood pressure (MAP; mmHg) responses to successive $\alpha_1$-, $\beta$-adrenergic, muscarinic and nicotinic receptor blockade (protocol II) in the conscious (panel *A*) and anesthetized (panel *B*) state in the same dog as in Fig. 1. The $\alpha_1$-adrenoceptors were blocked with doxazosin. Vagal cardiac acceleration is represented by the shaded area.

Following additional nicotinic receptor blockade, however, HR stabilized at a significantly lower level in the conscious state. As a consequence, the difference between the maximum HR after muscarinic receptor blockade and the minimum HR after nicotinic receptor blockade, *i.e.* maximum VCA, was significantly larger in the conscious state. In $\alpha_1$- and $\beta$-adrenoceptor blocked dogs, HR reached a lower maximum level after muscarinic receptor blockade and stabilized at a significantly lower level after nicotinic receptor blockade in the conscious state compared to the anesthetized state. Although the HR responses differed considerably, maximum VCA was comparable in the conscious and anesthetized states.

When in the conscious state the muscarinic receptors were blocked in $\alpha_1$- and $\beta$-adrenoceptor blocked dogs, the HR increased to a significantly lower maximum level than in $\beta$-adrenoceptor blocked dogs. Since the HR stabilized at a comparable minimum level after subsequent nicotinic receptor blockade, maximum VCA was significantly reduced in the presence of the $\alpha_1$-adrenoceptor antagonist (Fig. 1*A* & 2*A*). In contrast, in the anesthetized state maximum VCA was not affected by the $\alpha_1$-adrenoceptor antagonist; the HR increased to a similar maximum level following muscarinic receptor blockade and stabilized at a similar minimum level after nicotinic receptor blockade as observed in the $\beta$-adrenoceptor blocked dogs (Fig. 1*B* & 2*B*).

Although the maximum level to which HR increased following muscarinic receptor blockade varied greatly between dogs within the protocol I experiments, each dog showed a comparable maximum level following muscarinic receptor blockade in each of the protocol I experiments; a dog that exhibited a large response in the conscious state, displayed a similar response in anesthesia (Fig. 1*A* & *B*). It seems that the HR response to muscarinic receptor blockade is characteristic for each dog. In the $\alpha_1$- and $\beta$-adrenoceptor blocked anesthetized dogs the HR increased to a similar level as found in the protocol I experiments (Fig. 2*B*). In contrast, when the $\alpha_1$- and $\beta$-adrenoceptor blocked dogs were conscious, the HR increased to a considerably lower level (Fig. 2*A*).

The finding that the HR stabilized at a significantly higher minimum level after combined adrenergic, muscarinic and nicotinic receptor blockade in anesthesia than in the corresponding experiment in the conscious state was rather surprising, because this combined receptor blockade is considered to eliminate the autonomic nervous control of the HR and it was, therefore, expected that in the conscious and anesthetized conditions the HR would stabilize at a comparable level, *i.e.* the denervated heart rate (5). The higher HR after the nicotinic blockade in anesthesia cannot simply be explained as being a direct influence of the anesthetics, because, when nicotinic receptor blockade was replaced by vagal nerve transection, HR decreased to a level that matched that found after nicotinic receptor blockade in the conscious dogs (28). Therefore we conclude that in the anesthetized state a vagal cardiac accelerator influence persisted even after blocking adrenergic, muscarinic and nicotinic receptors. Hence, the combination of $\beta$-adrenergic, muscarinic and nicotinic receptor blockade is not the functional equivalent of surgical cardiac denervation. Our findings are in accordance with data in the literature. After eliminating autonomic nervous control pharmacologically, HR varied between 128 and 150 beats/min in anesthetized dogs (5, 10) and between 104 and 125 beats/min in conscious dogs (10, 33), whereas after eliminating autonomic nervous control surgically, HR varied between 84 and 120 beats/min (9, 10, 17, 25, 34).

The dissimilarities in the HR responses to muscarinic and nicotinic receptor blockade in the conscious and anesthetized states probably relate to the dissimilar responses in MAP to the receptor blockades. In the anesthetized state, the MAP responses to muscarinic and nicotinic receptor blockade were comparable in the $\beta$-adrenoceptor blocked and the $\alpha_1$- and $\beta$-adrenoceptor blocked dogs; following muscarinic receptor blockade, a marked and prolonged increase in MAP was observed, which reached a comparable maximum level; following nicotinic receptor blockade, MAP decreased to the same extent and stabilized at the same level. In the $\beta$-adrenoceptor blocked conscious dogs, however, a transient increase in MAP was observed following muscarinic receptor

blockade, which was significantly smaller than in the anesthetized state, and a prolonged decrease in MAP was observed following nicotinic receptor blockade, which was significantly larger than in the anesthetized state. In the $\alpha_1$- and $\beta$-adrenoceptor blocked conscious dogs, a transient increase in MAP was observed following muscarinic receptor blockade, which was significantly smaller than in the anesthetized state and somewhat smaller than in the $\beta$-adrenoceptor blocked conscious dogs. Furthermore this increase in MAP was, unlike in the $\beta$-adrenoceptor blocked conscious dogs, followed by a brief decrease. Following nicotinic receptor blockade, MAP decreased to the same extent and ultimately stabilized at the same level as found in the $\beta$-adrenoceptor blocked conscious dogs. That neuroleptanesthesia influences blood pressure regulation is not only inferred from the finding that MAP responded to muscarinic and nicotinic receptor blockade in the anesthetized and conscious state in a different way but also from the lack of a response to $\alpha_1$-adrenoceptor blockade in anesthesia.

Rigel and co-workers (27) have reported that the vagal cardiac accelerator system is under baroreflex control; a decrease in MAP reflexly induces a decrease in maximum VCA and conversely. Analogously, the increase in MAP following muscarinic blockade in the $\beta$-adrenoceptor blocked conscious dogs may have induced a pronounced increase in HR, *i.e.* a large VCA, and in the $\alpha_1$- and $\beta$-adrenoceptor blocked conscious dogs, the somewhat smaller increase and the subsequent brief decrease in MAP may have caused an attenuated HR increase, *i.e.* a reduced VCA. In the anesthetized state the sensitivity of this baroreflex is probably blunted, because a significantly larger increase in MAP in protocol I and II did not result in a higher maximum heart rate. A clear sign of the influence of anesthesia on the blood pressure was that MAP did not respond to $\alpha_1$-adrenoceptor blockade and the subsequent response to muscarinic blockade remained unchanged in comparison with $\beta$-adrenoceptor blocked anesthetized dogs. As a consequence, the HR response remained unchanged as well. Similar results have been described by Brunsting and co-workers (5). In addition, Schuil (31) showed that when the dogs are in neuroleptanesthesia, no reflex responses in VCA are observed in response to angiotensin II- and sodiumnitroprusside-induced changes in blood pressure.

After blocking the nicotinic receptors the HR stabilized at a significantly higher level in anesthesia than in the conscious state, signifying a larger residual VCA in anesthesia. This dissimilarity probably also relates to differences in the accompanying decrease in MAP. MAP decreased to a larger extent and ultimately stabilized at a lower level in the conscious state and, consequently, the vagal accelerator tone reflexly decreased significantly more than in anesthesia. Because in the conscious state HR stabilized at a level comparable to that found after vagotomy, (28), the vagal tone has

almost been annulled and HR has approximated its denervated value. In contrast, MAP is still elevated after nicotinic blockade in anesthesia and the vagal accelerator influence on HR endured. That the vagal accelerator influence remains even after the combined adrenergic, muscarinic and nicotinic receptor blockade indicates the existence of non-adrenergic, non-cholinergic transmission not only in the target organ but also in the ganglion. This is in keeping with the excitatory postsynaptic potentials observed in response to preganglionic nerve stimulation in the presence of a nicotinic and a muscarinic receptor antagonist (1, 18).

How neuroleptanesthesia affects the circulation is difficult to unravel, because each component of the neuroleptanesthesia, the neuroleptic agent fluanisone, the analgetic agent fentanyl, nitrous oxide, and the neuromuscular blocking agent vecuronium have their own specific effects, and each of them may influence the others. Fentanyl, fluanisone and vecuronium have been described to enhance vagal tone (2, 15, 16, 21, 22, 29). Furthermore, fentanyl and fluanisone attenuate the sympathetic tone (21, 22) and possess $\alpha$-adrenoceptor blocking actions, which result in moderate hypotension (21, 22, 23, 24, 32), and increase the release of catecholamines (8, 12). Moreover, fentanyl and fluanisone may influence the cardiovascular system through opioid and dopamine receptors which have been found in the membrane of ganglion cells (8, 11, 14, 15, 19, 23). Nitrous oxide, administered in combination with fentanyl and fluanisone, thereby converting neuroleptanalgesia to neuroleptanesthesia, is described to posses inotropic and pressor effects, and to modulate cardiac output and baroreflex sensitivity (8, 15, 20, 26).

In conclusion, the present study highlights the importance of using trained chronically instrumented conscious dogs for investigating the vagal cardiac accelerator system, especially in relation to blood pressure regulation, because experiments conducted in neuroleptanesthesia leads to controversial results and erroneous conclusions.

## References

1. Bachoo M, Isacoff E, Polosa C (1988) Slow cardioacceleration mediated by noncholinergic transmission in the stellate ganglion of the cat. *Can J Physiol Pharmacol* 66:1066-1074
2. Baldessarini RJ (1992) Drugs and the treatment of psychiatric disorders. In: Goodman Gilman A, Rall TW, Nies AS, Taylor P (Eds) *The pharmaceutical basis of therapeutics.* McGraw-Hill p. 383-435
3. Brouha L, Nowak SJG (1939) The role of the vagus in the cardio-accelerator action of atropine in sympathectomized dogs. *J Physiol* 95:439-453
4. Brunsting JR, Bennekers JH, Schuil HA, Zijlstra WG (1979) Incomplete cardiac vagal blockade with atropine in the anesthetized dog. *Pflügers Arch* 381:293-295
5. Brunsting JR, Schuil HA, Zijlstra WG (1983) Intrinsic heart rate in the dog determined by

pharmacologic denervation. *Am J Physiol* 245:H592-H597

6. Brunsting JR, Patberg WR, Schuil HA, Zijlstra WG (1985) Effect of autacoid antagonists on vagal cardioacceleration in the dog. *J Physiol* 36:39
7. Chassaing C, Boucher M, Breysse C, Duchêne-Marullaz P (1992) Contribution of vagal blockade to the tachycardia induced by the antimuscarinic agents atropine and pirenzepine. *J Auton Pharmacol* 12:359-368
8. De Castro J, Van de Water A, Wouters L, Xhonneux R, Reneman R, Kay B (1979) Comparative study of cardiovascular, neurological and metabolic side effects of eight narcotics in dogs. *Acta Anaesthesiol Belg* 30:5-99
9. Donald DE, Samueloff SL, Ferguson D (1967) Mechanisms of tachycardia caused by atropine in conscious dogs. *Am J Physiol* 212:901-910
10. Evans JM, Randall DC, Funk JN, Knapp CF (1990) Influence of cardiac innervation on intrinsic heart rate in dogs. *Am J Physiol* 258:H1132-H1137
11. Feuerstein G, Sirén A (1987) The opioid system in cardiac and vascular regulation of normal and hypertensive states. *Circulation* 75:125-129
12. Foucart S, Lacaille-Belanger P, Kimura T, Nadeau R, De Champlain J (1988) Modulation of adrenal catecholamine release by $DA_2$ dopamine receptors in the anaesthetized dog. *Clin Exp Pharmacol Physiol* 15:601-611
13. Freeman NE, Page IH (1937) Hypertension produced by constriction of the renal artery in sympathectomized dogs. *Am Heart J* 14:405-414
14. Freye E, Arndt JO (1979) Perfusion of the fourth cerebral ventricle with fentanyl induces naloxone reversible bradycardia, hypertension and EEG synchronization in conscious dogs. *Naunyn-Schmiedeberg's Arch Pharmacol* 307:123-128
15. Holaday JW (1983) Cardiovascular effects of endogenous opiate systems. *Annu Rev Pharmacol Toxicol* 23:541-594
16. Inoue K, Samodelov LF, Arndt JO (1980) Fentanyl activates a particular population of vagal efferents which are cardioinhibitory. *Naunyn-Schmiedeberg's Arch Pharmacol* 312:57-61
17. Kaczmarczyk G, Schmidt E (1990) Sodium homeostasis in conscious dogs after chronic cardiac denervation. *Am J Physiol* 258:F805-F811
18. Katayama Y, Nishi S (1986) Peptidergic transmission In: Karczmar AG, Koketsu K, Nishi S (Eds) *Autonomic and enteric ganglia; Transmission and its Pharmacology*. Plenum Press, New York and London, p. 181-200
19. Koyanagawa H, Musha T, Kanda A, Kimura T, Satoh S (1989) Inhibition of vagal transmission by cardiac sympathetic nerve stimulation in the dog: possible involvement of opioid receptor. *J Pharmacol Exp Ther* 250:1092-1096
20. Krahwinkel DJ, Sawyer DC, Eyster GE, Bender G (1975) Cardiopulmonary effects of fentanyl droperidol, nitrous oxide, and atropine sulfate in dogs. *Am J Vet Res* 36:1211-1219
21. Laubie M, Schmitt H, Drouillat M (1977) Central sites and mechanisms of the hypotensive and bradycardic effects of the narcotic analgesic agent fentanyl. *Naunyn-Schmiedeberg's Arch Pharmacol* 296:255-261
22. Laubie M, Schmitt H (1980) Action of the morphinomimetic agent, fentanyl, on the nucleus tractus solitarii and the nucleus ambiguus cardiovascular neurons. *Eur J Pharmacol* 67:403-412
23. Leysen JE, Niemegeers CJE (1985) Neuroleptics. In: Lajtha A (Ed) *Handbook of Neurochemistry* vol 9. Alterations of metabolites in the nervous system; Plenum Press New York, p. 331-361
24. Niemegeers CJE, Janssen PAJ (1979) A systematic study of the pharmacological activities of

dopamine antagonists. *Life Science* 24:2201-2216

25. Otani N, Yang T, Levy MN (1993) Intense sympathetic stimulation releases neuropeptide Y but fails to evoke sustained coronary vasoconstriction in dogs. *Circ Res* 72:816-826
26. Philbin DM, Foex P, Drummond G, Ryder WA, Jones LA (1984) Regional ventricular function with sufentanyl anesthesia: The effect of nitrous oxide. *Anesth Analg* 63:260
27. Rigel DF, Lipson D, Katona PG (1984) Excess tachycardia: heart rate after antimuscarinic agents in conscious dogs. *Am J Physiol* 246:H168-H173
28. Roossien A, Brunsting JR, Zaagsma J, Zijlstra WG (1993) Further evidence for non-cholinergic ganglionic transmission in the vagal cardioaccelerator pathway in dogs. *Abstract of Congress XXXII International Union of Pysiological Sciences*, Glasgow
29. Salmenperä M, Peltola K, Takkunen O, Heinonen J (1983) Cardiovascular effects of pancuronium and vecuronium during high dose fentanyl anesthesia. *Anesth Analg* 62:1059-1064
30. Schuil HA, Brunsting JR, van der Molen H, Zijlstra WG (1981) Cardioacceleratory effect of muscarinic blocking agents in the dog. *Eur J Pharmacol* 69:229-233
31. Schuil HA (1987) *Vagale cardio-acceleratie en denervatie van het hondehart.* Thesis, University of Groningen, Van Denderen, Groningen, The Netherlands
32. Toda N, Hatano Y (1977) Alpha-adrenergic blocking action of fentanyl on the isolated aorta of the rabbit. *Anesthesiology* 46:411-416
33. Woodman OL, Vatner SF (1987) Coronary vasoconstriction mediated by $\alpha_1$- and $\alpha_2$-adrenoceptors in conscious dogs. *Am J Physiol* 253:H388-H393
34. Yoshitaka H, Furuya F, Morita H, Nishida H, Hosomi H, Matsushita K, Tanaka S (1991) Effect of exercise on fractional extraction of catecholamines by the denervated heart in conscious dogs. *Jpn J Physiol* 41:749-757

# 4

# REFLEX CONTROL OF THE VAGAL CARDIAC ACCELERATOR SYSTEM IN CONSCIOUS DOGS


## Abstract

We determined the effects of pharmacologically induced changes in mean arterial blood pressure (MAP) on the vagal cardiac accelerator (VCA) activity in $\beta$-adrenoceptor blocked (timolol 0.2 mg/kg and 0.2 mg/kg/h, i.v.) conscious dogs. When MAP was increased by $\alpha_1$-adrenoceptor stimulation (methoxamine 0.1 mg/kg, i.v.) or vasopressin (10 mU/kg, i.v.), the heart rate (HR) increased ($33\pm12$ and $26\pm6$ beats/min) when muscarinic receptors had been blocked (methylatropine 0.5 mg/kg and 0.5 mg/kg/h, i.v.), signifying reflex activation of the VCA system. In reverse, when MAP was decreased, following the infusion of sodiumnitroprusside (150 $\mu$g and 100 $\mu$g/min, i.v.) VCA activity decreased ($30\pm7$ beats/min).

When methoxamine and vasopressin were injected before muscarinic receptor blockade, bradycardia was observed. However, the tachycardia in response to subsequent muscarinic receptor blockade was enhanced, reaching a maximum HR of $218\pm10$ and $211\pm10$, respectively, versus $196\pm15$ beats/min in the control experiments, thereby signifying augmented VCA activity. Conversely, when MAP was decreased due to $\alpha_1$-adrenoceptor blockade (doxazosin 0.1 mg/kg, i.v.) reflex tachycardia was observed ($20\pm2$ beats/min). Following subsequent muscarinic receptor blockade, an attenuated increase in HR to a level of $171\pm13$ beats/min was observed, signifying an attenuated VCA activity.

Although the vagal cardiac accelerator and decelerator systems exhibit opposite chronotropic effects, they seem both to be activated in response to elevations in MAP. Our experiments indicate the participation of the VCA system in the baroreflex control of the heart rate.

## Introduction

Numerous investigations on the relationship between blood pressure and heart rate have established the importance of the baroreflex control of the circulation (13, 14). Traditionally, only the relative importance of the sympathetic cardiac accelerator and vagal cardiac decelerator systems have been investigated in relation to chronotropic adjustments to blood pressure alterations (23, 28, 31, 32). The contribution of the vagal cardiac accelerator (VCA) mechanism has generally been disregarded, because its existence is not commonly acknowledged.

Jourdan and Morin (19) were the first who described the reflex activation of the VCA system following afferent vagal nerve stimulation. More recently, Rigel and co-workers (26) confirmed the relationship by altering blood pressure in conscious dogs with blocked $\beta$-adrenergic and muscarinic receptors. They observed a reflexly induced increase in heart rate in response to an increase in blood pressure, and conversely. The involvement of the VCA system in the baroreflex, however, remained controversial because the reflex was not encountered in response to blood pressure alterations or to afferent nerve stimulation in numerous other studies (5, 10, 20, 30, 31). Moreover, Rigel et al. (26) reported that a VCA reflex response did not occur in every dog and that in dogs that did display the VCA reflex response, the ability to respond to a phenylephrine-induced rise in blood pressure is lost approximately 2h after the administration of atropine. Therefore, to elucidate the role of the VCA system in the baroreflex mechanism, we studied the chronotropic responses to pharmacologically induced blood pressure alterations in conscious dogs.

## Methods

*Surgical preparations*

The experiments were performed on 5 adult mongrel dogs of both sexes (25-36 kg). Three indwelling catheters (Tygon microbore S-54-HL, ID 0.5 mm, OD 1.5 mm) were inserted into the right omocervical vein and one catheter in the omocervical artery and the tips were advanced to the superior caval vein and descending aorta. Subsequently, the catheters were tunnelled subcutaneously and secured to the skin in the midscapular region. This procedure was carried out under neuroleptanesthesia with fentanyl (0.1 mg/kg, i.m.) and fluanisone (5 mg/kg, i.m.) (Hypnorm, Janssen). Catheters were kept patent by daily flushing with heparinized saline (2000 U/ml). The dogs wore jackets to protect the catheters from being chewed on. The first experiment was carried out after at

least 14 days. The study protocol was approved by the appropriate Animal Care and Use Committee (Groningen University, FDC 65-0186).

*Experimental protocol*

The dogs were submitted to nine experiments, separated by an interval of at least 14 days. Before experiments in the conscious state were performed, the dogs were habituated to the laboratory surroundings and personnel, and trained to stand quietly on a padded table in a modified Pavlovian sling. To minimize external influences that might excite the animals, the room was dimly lit and the sound level kept as low as possible. In each experiment, the $\beta$-adrenergic and muscarinic receptors were blocked at 15 min and 45 min after the start of the experiment, respectively. In the control experiment (protocol I) the VCA activity was observed for a 2-h period and subsequently abolished by blocking the ganglionic nicotinic receptors. In the following experiments, the influence of changes in mean arterial blood pressure (MAP), induced after (protocol II) or before and after (protocol III) the muscarinic receptor blockade, on the activity of the VCA system was examined. In protocol II experiments, MAP was either increased, with the $\alpha_1$-adrenergic receptor agonist methoxamine (protocol II-*A*) or with vasopressin (protocol II-*B*), or decreased, with sodiumnitroprusside (protocol II-*C*). The increase in MAP was induced 30 min after muscarinic receptor blockade, when VCA had typically declined to about 50% of its maximum level (26). The decrease in MAP was induced 15 min after muscarinic receptor blockade, when VCA typically attained its maximum level (26). In protocol III experiments we studied the influence on HR of changes in MAP induced before and after muscarinic receptor blockade. MAP was increased by methoxamine (protocol III-*A*) or vasopressin (protocol III-*B*), and decreased by sodiumnitroprusside (protocol III-*C*) or doxazosin, an $\alpha_1$-adrenergic receptor antagonist (protocol III-*D*), 15 min before muscarinic receptor blockade. In the vasopressin and doxazosin premedicated animals MAP was increased 75 and 30 min after muscarinic receptor blockade, using methoxamine (protocol III-*B*) and vasopressin (protocol III-*D*), respectively. In the methoxamine and nitroprusside treated animals MAP was decreased 15 min after muscarinic receptor blockade, using nitroprusside (protocol III-*A* and III-*C*, respectively). To ascertain that the chronotropic responses were indeed reflexly induced, an additional experiment was performed in three vagotomized conscious dogs in which vasoactive agents were injected after combined $\beta$-adrenergic and muscarinic receptor blockade (protocol IV).

*Circulatory measurements*

Ag/AgCl electrodes (Quinton) were attached to the shaved legs, to obtain a lead II electrocardiogram (ECG). HR was determined from the ECG with a cardiotachometer (ECG module, Philips V 692). MAP was measured through the implanted catheter in the aorta and measured with a strain-gauge manometer (Uniflow, Baxter). The signals were amplified, analog-to-digital converted, and fed into a computer for data processing and display. The data were also registered on chart recorders (Kipp BD 101; Elema Mingograph).

*Drugs*

The $\beta$-adrenergic receptors were blocked with timolol (0.2 mg/kg and 0.2 mg/kg/h) (Sigma). Methylatropine (0.5 mg/kg and 0.5 mg/kg/h) (Sigma) was used as a muscarinic receptor blocking agent. In the control experiments ganglionic nicotinic receptor blockade was achieved by hexamethonium (HEX) (20 mg/kg) (Fluka). The $\alpha_1$-adrenergic receptor agonist methoxamine (MET) (0.1 mg/kg) (Wellcome) and vasopressin (VAS) (10 mU/kg) (Sandoz) were administered to increase MAP. MAP decreases were induced by sodiumnitroprusside (NIP) (150 $\mu$g and 100 $\mu$g/min, during 4 min) (Roche) and the $\alpha_1$-adrenergic receptor antagonist doxazosin (DOX) (0.1 mg/kg) (Pfizer). All drugs were dissolved in an NaCl solution (154 mmol/l) and administered intravenously.

*Data analysis*

The maximum VCA activity was quantified by subtracting the minimum HR after combined $\beta$-adrenergic and muscarinic receptor blockade from the maximum HR after this combined receptor blockade. The minimum HR, which was mostly observed after additional ganglionic nicotinic receptor blockade, was taken as a reference, because it is closest to the denervated HR. In case vagotomy had been performed, the minimum HR was considered to be the denervated HR. $\Delta f_X max$ and $\Delta p_X max$ refer to the maximum changes in HR and MAP in response to intervention X, respectively. Values are reported as means $\pm$ S.E.M. Results were analyzed using Student's paired *t*-test. A $P$ value $\leq$ 0.05 was considered significant.

**Results**

The HR and blood pressure responses to the pharmacological interventions in protocol I and II experiments are presented in Table 1. In the control experiment (protocol I), HR increased to a maximum of 196 beats/min following muscarinic receptor

blockade and declined to 146 beats/min during the 2-h observation period. Following the ganglionic nicotinic blockade HR decreased by 18 beats/min and stabilized at 128 beats/min. As a consequence, the maximum VCA activity amounted to 68 beats/min. Between the dogs there was a large variation in maximum VCA activity ranging from 27 to 92 beats/min. In response to muscarinic receptor blockade, MAP increased by 24 mmHg. Following nicotinic receptor blockade MAP decreased by 55 mmHg. In protocol II experiments HR and MAP responses to muscarinic receptor blockade resembled those found in protocol I experiment (Table 1; Fig. 1). Since each dog displayed the same results as in protocol I experiment, the same variation in response to muscarinic receptor blockade was observed. The response, therefore, is characteristic for each dog. In Fig. 2 a protocol II-*B* experiment is shown in which the VCA activity, uncovered after β-adrenergic and muscarinic receptor blockade, reflexly increased through a vasopressin-induced increase in MAP. The VCA activity is represented by the shaded area. The denervated HR of this dog (84 beats/min) and that of two other dogs were determined in separate experiments in which β-adrenergic and muscarinic receptors were blocked after vagotomy had been performed on the previous day.

Table 1 Heart rate (beats/min) and mean arterial blood pressure (mmHg) responses to muscarinic receptor blockade and to either subsequent ganglionic nicotinic receptor blockade (protocol I) or to additional administration of vasoactive agents (protocol II) in β-adrenoceptor blocked conscious dogs.

| Protocol | I | II-*A* | II-*B* | II-*C* |
|---|---|---|---|---|
| reaction to muscarinic block | | | | |
| f[0] | 73±7 | 70±6 | 72±6 | 77±6 |
| fmax | 196±15 | 201±16 | 191±16 | 196±19 |
| Δpmax | 24±6 | 33±5 | 34±6 | 31±4 |
| intervention after muscarinic block | HEX | MET | VAS | NIP |
| f[0] | 146±9 | 182±14 | 175±15 | 184±16 |
| fmax/min | 128±7 | 215±7 | 201±11 | 154±9 |
| Δfmax | —18±2 | 33±12 | 26±6 | —30±7 |
| Δpmax | —55±7 | 97±2 | 72±11 | —51±7 |

Value are means ± S.E.M., $n=5$. f[0] symbolizes HR prior to the pharmacological intervention. fmax/min is the maximum HR after administration of MET and VAS or the minimum HR after HEX and NIP. Δfmax and Δpmax is the maximum change in HR and MAP in response to the intervention, respectively. HEX, MET, VAS, NIP stand for intravenous administration of hexamethonium, methoxamine, vasopressin and sodiumnitroprusside, respectively.

The methoxamine- and vasopressin-induced MAP increase of 97 and 72 mmHg was accompanied by an increase in HR of 33 and 26 beats/min, respectively (Table 1; Fig. 3). These responses were most prominent in dogs that displayed a moderate VCA activity following muscarinic receptor blockade. In response to the nitroprusside-induced MAP decrease of 51 mmHg, HR decreased 30 beats/min (Table 1; Fig. 3). This response was most noticeable in dogs that exhibited a large VCA activity. After termination of the nitroprusside infusion, MAP and HR returned to their previous levels.

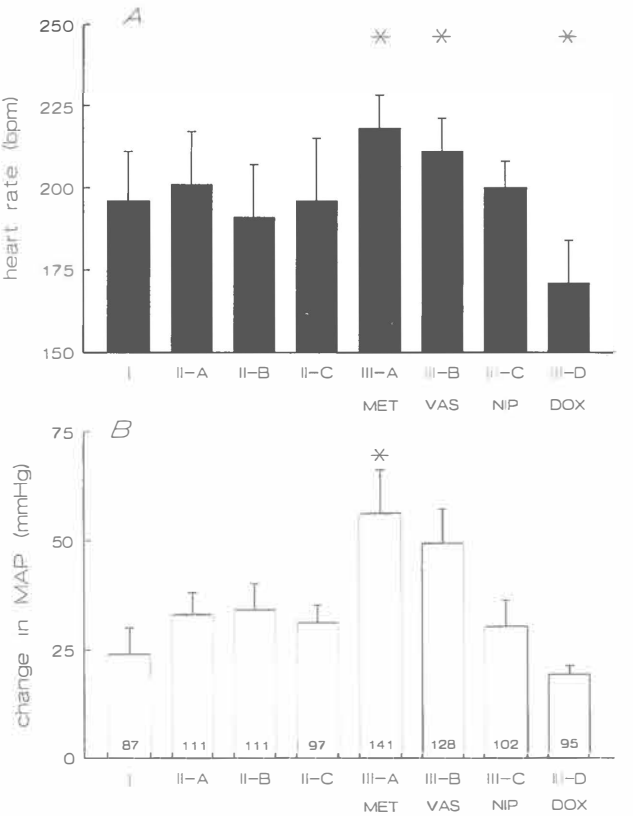



Fig. 1 Heart rate (beats/min) and change in mean arterial blood pressure (MAP; mmHg) following muscarinic receptor blockade. In Fig. 1*A* the maximum HR following combined $\beta$-adrenergic and muscarinic receptor blockade in protocol I, II and III experiments is presented. Protocol II is divided in groups of dogs ($n=5$) that were treated with either methoxamine (MET), vasopressin (VAS) or nitroprusside (NIP) *after* muscarinic receptor blockade and protocol III is divided in groups that were treated with MET, VAS, NIP or doxazosin (DOX) *before* muscarinic receptor blockade. In Fig. 1*B* the maximum changes in MAP in response to muscarinic receptor blockade in protocol I, II and III is displayed. Within the bars MAP prior to the intervention is reported. $n=5$; * $P \leq 0.05$ different from protocol I, Student's paired $t$-test.

When methoxamine or vasopressin was applied (protocol III-*A* and III-*B*, respectively) 15 min before muscarinic receptor blockade, MAP increased by 86 and 51 mmHg and was accompanied by a fall in HR of 53 and 30 beats/min, respectively (Table 2; Fig. 4). After methylatropine treatment HR increased to maximum levels that were significantly higher than obtained in the control experiment (218 and 211 vs. 196 beats/min) (Fig. 1*A*). These increases in HR were accompanied by larger increases in MAP (56 and 49 versus 24 mmHg) (Fig. 1*B*). When MAP was decreased by doxazosin preceding the muscarinic receptor blockade (protocol III-*D*), a reflex tachycardia of 20 beats/min was observed (Table 2; Fig. 4). Following subsequent muscarinic receptor blockade, HR increased to a significantly lower maximum level (171 beats/min) in comparison with the control experiments (Fig. 1*A*). When sodiumnitroprusside was infused preceding muscarinic receptor blockade (Protocol III-*C*), MAP fell by 37 mmHg and HR increased by 41 beats/min (Table 2; Fig. 4).

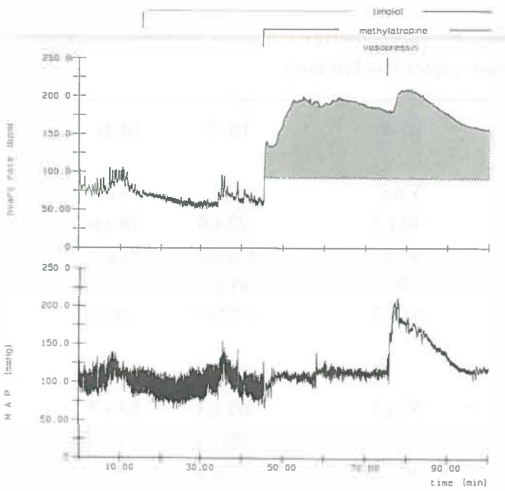



Fig. 2 Protocol II-*B* experiment: heart rate (beats/min) and mean arterial blood pressure (MAP; mmHg) responses to β-adrenergic and muscarinic receptor blockade and to subsequent administration of vasopressin. Vagal cardiac accelerator activity is represented by the shaded area. The denervated HR (84 beats/min) was determined in a separate experiment by β-adrenergic and muscarinic receptor blockade after vagotomy on the previous day. Note that after MAP is increased, using vasopressin (10 mU/kg, i.v.), HR increased rather than decreased, thereby indicating an increase in VCA activity.

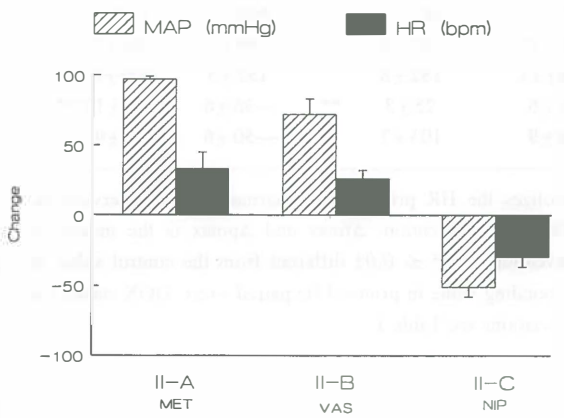



Fig. 3 Maximum changes in heart rate (HR; beats/min) induced by changes in mean arterial blood pressure (MAP; mmHg) *after* muscarinic receptor blockade in β-adrenoceptor blocked dogs ($n$=5) in protocol II. An increase in HR was induced through an increase in MAP, using methoxamine (MET) or vasopressin (VAS); a decrease in HR was induced through a decrease in MAP, using nitroprusside (NIP).

After the infusion had been stopped, both parameters returned to their previous levels. The subsequent muscarinic receptor blockade induced changes in HR and MAP which were comparable to those in protocol I and II. Additional administration of nitroprusside to the methoxamine pretreated, muscarinic receptor blocked dogs (protocol III-*A*) induced a decrease in MAP of 78 mmHg (Table 2). The resulting bradycardic response amounted to 31 beats/min and was comparable with that found following nitroprusside administration in protocol II.

In vasopressin-pretreated dogs the methoxamine-induced large increase in MAP (103 mmHg) resulted in an increase in HR that was somewhat less than found in protocol II experiment (25 versus 33 beats/min) (Table 2: protocol III-*B*). The vasopressin-induced MAP increase of 85 mmHg in dogs pretreated with doxazosin, caused a significantly larger increase in HR than obtained in the corresponding protocol II experiment (44 versus 26 beats/min) (Table 2: protocol III-*D*).

Table 2 Heart rate (beats/min) and mean arterial blood pressure (mmHg) responses to vasoactive agents before and after muscarinic receptor blockade in $\beta$-adreno- ceptor blocked dogs.

| protocol | III-*A* | | III-*B* | | III-*C* | III-*D* | |
|---|---|---|---|---|---|---|---|
| intervention before muscarinic block | MET | | VAS | | NIP | DOX | |
| f[0] | 82±7 | | 70±7 | | 72±6 | 78±6 | |
| fmax/min | 29±7 | | 40±6 | | 113±5 | 98±5 | |
| Δfmax | —53±5 | | —30±5 | | 41±7 | 20±2 | |
| Δpmax | 86±6 | | 51±7 | | —37±7 | —21±3 | |
| reaction to muscarinic block | | | | | | | |
| f[0] | 61±5 | * | 53±5 | | 63±3 | 83±7 | |
| fmax | 218±10 | * | 211±10 | * | 200±8 | 171±13 | * |
| Δpmax | 56±10 | * | 49±8 | | 30±6 | 19±2 | |
| intervention after muscarinic block | NIP | | MET | | NIP | VAS | |
| f[0] | 199±15 | | 157±8 | | 188±9 | 156±11 | |
| fmax/min | 168±13 | | 182±8 | | 152±5 | 200±6 | |
| Δfmax | —31±6 | | 25±3 | ** | —36±6 | 44±10 | ** |
| Δpmax | —78±9 | | 103±7 | | —50±6 | 85±9 | |

Values are means ± S.E.M., $n$= 5. f[0] symbolizes the HR prior to the pharmacological intervention. fmax/min is the maximum or minimum HR after the intervention. Δfmax and Δpmax is the maximum change in HR and MAP in response to the intervention. * $P \leq 0.05$ different from the control value in protocol I, ** $P \leq 0.05$ different from the corresponding value in protocol II, paired $t$-test. DOX stands for intravenous injection of doxazosin; for other abbreviations see Table 1.

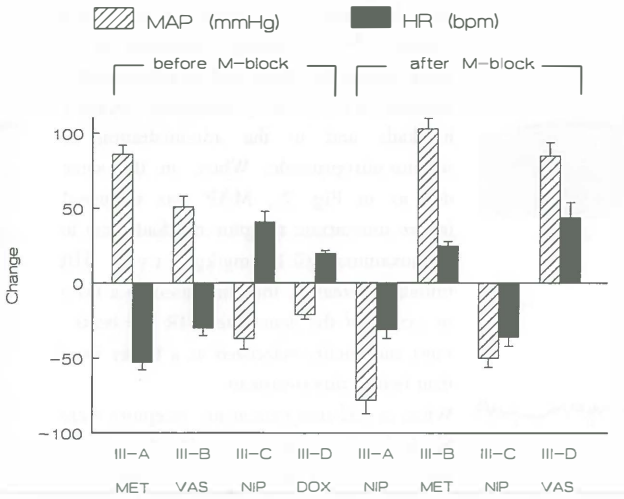



Fig. 4 Maximum changes in heart rate (HR; beats/min) induced by changes in mean arterial blood pressure (MAP; mmHg) *before* and *after* muscarinic receptor blockade in $\beta$-adrenoceptor blocked dogs ($n$=5) in protocol III. *Before* muscarinic receptor blockade (M-block), an increase in MAP due to methoxamine (MET) (protocol III-*A*) or vasopressin (VAS) (protocol III-*B*), elicited a decrease in HR, whereas a decrease in MAP due to nitroprusside (NIP) (protocol III-*C*) or doxazosin (DOX) (protocol III-*D*), resulted in an increase in HR. *After* muscarinic receptor blockade an increase in MAP due to MET (protocol III-*B*) or VAS (protocol III-*D*), elicited an increase in HR, whereas a decrease in MAP due to NIP (protocol III-*A* and III-*C*), resulted in a decrease in HR.

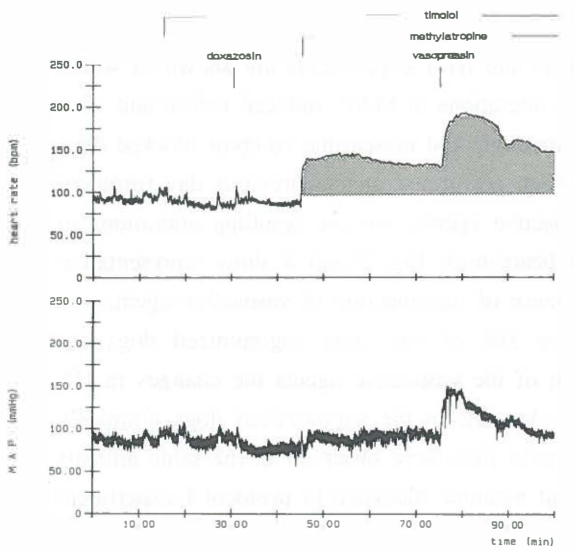



Fig. 5 Protocol III-*D*: heart rate (beats/min) and mean arterial blood pressure (MAP; mmHg) responses to $\beta$- and $\alpha_1$-adrenoceptor blockade followed by muscarinic receptor blockade and to vasopressin. After muscarinic receptor blockade a modest VCA activity was observed. When, in addition, MAP was increased *after* muscarinic receptor blockade using vasopressin (10 mU/kg, i.v.), HR and thereby VCA activity increased. Note that after $\beta$-adrenoceptor blockade in this animal, the $\alpha_1$-adrenoceptor antagonist doxazosin induces a decrease in MAP that is followed by an insignificant reflex tachycardia. This tachycardia can only be brought about by the decrease in vagal decelerator tone. In this animal, however, the vagal decelerator activity was already small before the injection of doxazosin, as is signified by the relatively high HR ($\pm$90 beats/min).

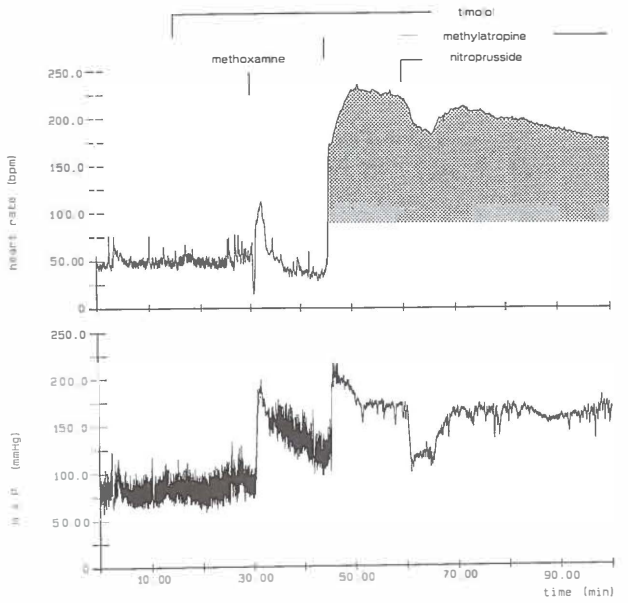



Fig. 6 Protocol III-*A* experiment: heart rate (beats/min) and mean arterial blood pressure (MAP; mmHg) responses to β-adrenoceptor blockade and $\alpha_1$-adrenoceptor stimulation followed by muscarinic receptor blockade and to the administration of sodium-nitroprusside. When, in the same dog as in Fig. 2., MAP was increased *before* muscarinic receptor blockade due to methoxamine (0.1 mg/kg, i.v.), HR initially decreased, then increased to a level in excess of the denervated HR (84 beats/-min) and finally stabilized at a lower level than before this treatment.
When in addition muscarinic receptors were blocked, the increase in MAP and the uncovered VCA activity were enhanced in comparison to Fig. 2. When MAP was decreased *after* muscarinic receptor blockade due to sodiumnitroprusside (150 μg and 100 μg/min, i.v., during 4 min), MAP and HR decreased during the infusion.

In Fig. 5 and 6 representative protocol III-*D* and III-*A* experiments are shown in which VCA activity was reflexly changed through alterations in MAP, induced before and after muscarinic receptor blockade. In three β-adrenergic and muscarinic receptor blocked dogs in which bilateral cervical vagotomy had been performed on the previous day (protocol IV), neither the administration of the vasoactive agents, nor the resulting alterations in MAP induced major changes in HR (<5 beats/min). Fig. 7 and 8 show representative protocol IV experiments in which the influence of the injection of vasoactive agents and the resulting blood pressure alterations on HR of conscious vagotomized dogs are displayed. Note that following the injection of the vasoactive agents the changes in HR are insignificant. Furthermore, it appeared that HR in the vagotomized dogs ultimately stabilized at lower levels (84 and 89 beats/min) than were observed in the same animals after combined β-adrenergic, muscarinic and nicotinic blockade in protocol I experiment (149 and 114 beats/min, respectively).

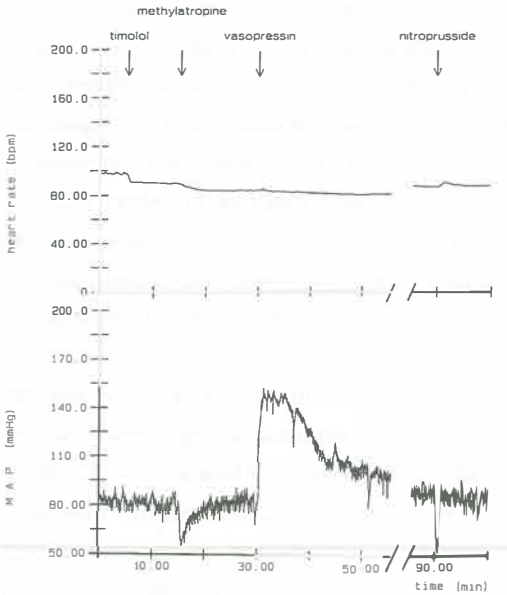



Fig. 7 Protocol IV experiment: heart rate (beats/min) and mean arterial blood pressure (MAP; mmHg) responses to β-adrenoceptor and muscarinic receptor blockade and subsequent administration of vasopressin (10 mU/kg, i.v.) and nitroprusside (150 μg, i.v.) in a conscious dog which had its vagal nerves transected the day before the experiment. Note that after β-adrenoceptor and muscarinic receptor blockade the HR stabilized at a level of 84 beats/min, *i.e.* the denervated HR, and that the administration of the vasoactive agents nor the resulting changes in MAP induced major changes in HR.

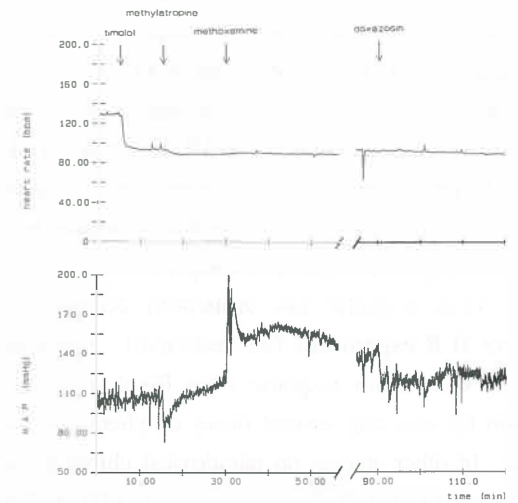



Fig. 8 Protocol IV experiment: heart rate (beats/min) and mean arterial blood pressure (MAP; mmHg) responses to β-adrenoceptor and muscarinic receptor blockade and subsequent administration of methoxamine (0.1 mg/kg, i.v.) and doxazosin (0.1 mg/kg, i.v.) in a conscious dog which had its vagal nerves transected the day before the experiment. Note that after β-adrenoceptor and muscarinic receptor blockade HR stabilized at a level of 89 beats/min, *i.e.* the denervated HR, and that the administration of the vasoactive agents nor the resulting changes in MAP induced major changes in HR.

## Discussion

Alterations in blood pressure are counteracted by baroreceptor reflex-mediated adjustments in autonomic nervous activity (14, 23). Stimulation of the baroreceptors typically induces opposite changes in sympathetic and vagal nerve activity, which underlie the inverse relationship between MAP and HR, as already observed by Marey in 1859.

In the present study, we established the contribution of the VCA system in the baroreflex control of HR. After blocking the sympathetic cardiac accelerator effect, using timolol, and the vagal cardiac decelerator effect, using methylatropine, the inverse chronotropic reaction to pharmacologically induced MAP changes was prevented and an apparently paradoxical relationship was revealed: a rise in MAP reflexly resulted in an increase in HR and conversely. These results are consistent with those obtained by others (12, 25, 26, 34), although, in some of these studies the relation with the VCA system was not recognized (12, 34), while in others the existence of the VCA system was ignored and the positive chronotropic response to hypertensive stimuli interpreted as an increase in intrinsic HR (25).

In several studies the apparently paradoxical chronotropic reflex response was not established (10, 30, 31). The absence of an accelerator response to angiotensin-induced rise in MAP after muscarinic receptor blockade, as observed by Donald and co-workers (10), may be explained by the elevations in MAP induced before atropine treatment, which may have already increased the vagal tone to its physiological ceiling, as indicated by the high heart rates after atropinization in their study. This latter explanation is in agreement with our findings: when in protocol III-*B* MAP was elevated before the muscarinic receptor blockade, the VCA activity unmasked after muscarinic receptor blockade was enhanced and when subsequently MAP was elevated after muscarinic receptor blockade, using methoxamine, the VCA reflex response appeared to be attenuated in comparison to the methoxamine-induced rise in MAP in protocol II. Conversely, when in protocol III-*D* MAP was reduced before muscarinic receptor blockade using doxazosin, VCA activity following muscarinic receptor blockade was attenuated in comparison with protocol I-*A* (Fig. 1*A*). When subsequently MAP was elevated using vasopressin, an enhanced VCA response (44 beats/min) occurred in comparison with the corresponding protocol II-*B* experiment (26 beats/min). A similar explanation may be given for the loss of the VCA reflex response when Rigel et al. (26) elevated the blood pressure again and again by injecting several doses of phenylephrine into their dogs within the same experiment. In other studies no paradoxical chronotropic change was noticed in response to alterations in MAP (30, 31). Vatner et al. (31) studied the reflex tachycardia following a reduction in MAP, which was induced by injecting nitroglycerine or by occluding the caval veins in conscious dogs. After $\beta$-adrenergic and muscarinic receptor blockade they did not observe major changes in HR in response to a decrement in MAP, despite the fact that the decrease in MAP was of the same magnitude as in our experiments. Their hypotensive stimuli, however, were only short-lasting (about 30 s), whereas in our study the hypotensive stimulus persisted for about 4 min. In other

studies the use of anesthesia may account for conflicting results, because the VCA activity and its reflex response appear to be suppressed during anesthesia (6, 8).

The lack of chronotropic responses to the vasoactive agents or to the resulting hypertensive and hypotensive stimuli in vagotomized dogs with blocked $\beta$-adrenergic and muscarinic receptors (present study) or in animals which had their vagi cooled (26) demonstrates that intact vagus nerves are necessary to generate chronotropic responses and confirms the involvement of the VCA system in the baroreflex. The lack of direct chronotropic properties of the vasoactive agents used has also been confirmed in other studies (12, 15, 28).

Since little variation in VCA activity was observed within each dog when the conditions were similar (protocol I and II), but changed when the physiological conditions were altered before the muscarinic receptor blockade (protocol III), the VCA activity possibly reflects the extent to which the vagal limb of the autonomic nervous system is at the moment involved in cardiovascular control. Hence, the VCA activity of a dog may give information on its autonomic status.

Although muscarinic receptor blockade unmasks the VCA system, its activity after muscarinic receptor blockade does not necessarily corresponds to the activity before the muscarinic receptor blockade, because the changes in MAP that follow the muscarinic receptor blockade may have modulated the activity simultaneously. The activation of a baroreflex following muscarinic receptor blockade is not only indicated by an increase in vagal activity, represented by the slowly developing increase in VCA activity reaching a maximum level after a few minutes, but is also suggested by a simultaneous decrease in sympathetic nervous activity as indicated by the decrease in catecholamine concentrations following muscarinic receptor blockade as observed by Roossien et al. (29).

Before muscarinic receptor blockade, the VCA response to elevations in MAP is obscured by the more rapidly developing vagal decelerator effect. The secondary tachycardia that follows the initial fall in HR after the methoxamine-induced elevation in MAP (Fig. 6) may be evoked by the VCA system, especially since HR reached levels in excess of the denervated HR of 84 beats/min, as was established in the vagotomized state. This overshoot reaction may as well be analogous with the rebound tachycardia that follows the bradycardia elicited by vagal nerve stimulation, designated post-vagal tachycardia (PVT) (9). Post-vagal tachycardia and vagal cardiac acceleration have much in common, since both arise from the release of an excitatory substance directly at the SA-node and neither of them is mediated by a sympathetic adrenergic mechanism. Nevertheless, according to Celler and Lovell (7, 24) post-vagal tachycardia and vagal cardiac acceleration are distinct phenomena with different causes, because the time course

of VCA differs from that of PVT. They also claim that PVT, unlike VCA, requires a functioning muscarinic receptor. Furthermore, VCA is considered to differ from PVT because high frequencies of electric vagal nerve stimulation, in excess of 10 Hz, are needed to elicit VCA activity, which, according to Celler and Lovell (7), is physiologically unrealistic, whereas PVT can be observed using low frequencies. Katona et al. (22), however, showed that the firing frequency of intact vagal efferent fibers ranges between 2-30 Hz and therefore VCA activity, as is revealed in response to high frequency vagal nerve stimulation (16), is of physiological significance.

The way in which the VCA system acts on pacemaker activity has not yet been untangled. The VCA activity has been brought in relation to vasoactive intestinal peptide (VIP), because VIP is co-localized with acetylcholine in the vagal nerve terminals (11, 33). Furthermore, VIP is released in response to high frequency vagal nerve stimulation similar to the frequency of stimulation that results in maximum VCA responses (16, 17). Finally, exogenous-VIP has been shown to bring on a prolonged tachycardia (21, 27).

The observation that a strong vagal nerve stimulation is necessary to elicit a forceful VCA effect, suggests the VCA system to be active during physiological states characterized by a low basal HR, notably during sleep, and thus to be a protective mechanism to avoid cardiac arrest. During physical and psychological stress situations, the VCA system probably participates in chronotropic control as well (4, 18). This hypothesis is corroborated by the increase in HR to a level far in excess of the denervated level in $\beta$-adrenoceptor blocked dogs during treadmill running (2, 3, 32); the increased HR was further enhanced after atropinization (2, 3). Versteeg et al. (32) showed that the exercise-induced increase in HR was inhibited during vagal cooling.

The VCA system may be involved in the development of cardiovascular dysfunction, because dogs with experimental heart failure showed a considerably lower HR following muscarinic receptor blockade than normal dogs (31), suggesting suppressed VCA activity. Further investigation of the VCA system may also be important since defective vagal control has been linked to hypertension (13), coronary artery disease (1) and healed myocardial infarction (2). The determination of VCA activity thus may become of diagnostic significance. In conclusion, we have demonstrated that the vagal cardiac accelerator system contributes to the chronotropic control of the heart, and that it partakes in the baroreceptor reflex.

## References


1. Airaksinen KE, Ikäheimo MJ, Linnaluoto MK, Niemelä M, Takkunen JT (1987) Impaired vagal heart rate control in coronary artery disease. *Br Heart J* 58:592-597
2. Billman GE, Hoskins RS (1989) Time-series analysis of heart variability during submaximal exercise: evidence for reduced cardiac vagal tone in animals susceptible to ventricular fibrillation. *Circulation* 80:146-157
3. Billman GE, Dujardin J-P (1990) Dynamic changes in cardiac vagal tone as measured by time-series analysis. *Am J Physiol* 258:H896-H902
4. Brouha L, Cannon WB, Dill DB (1936) The heart rate of the sympathectomized dog in rest and exercise. *J Physiol* 87:345-359
5. Brouha L, Dill DB, Nowak SJG (1937) Recherches sur l'accélération cardiaque chez le chien sympathectomisé. *C R Soc Biol* 126:909-910
6. Brunsting JR, Schuil HA, Zijlstra WG (1983) Intrinsic heart rate in the dog determined by pharmacologic denervation. *Am J Physiol* 245:H592-H597
7. Celler BG, Lovell NH (1991) Dynamics of cardiac period responses after prolonged vagal stimulation in the dog. *Ann Biomed Eng* 19:273-289
8. Chassaing C, Boucher M, Breysse C, Duchêne-Marullaz P (1992) Contribution of vagal blockade to the tachycardia induced by the antimuscarinic agents atropine and pirenzepine. *J Auton Pharmacol* 12:359-368
9. Copen DL, Cirillo DP, Vassalle M (1968) Tachycardia following vagal stimulation. *Am J Physiol* 215:696-703
10. Donald DE, Samueloff SL, Ferguson D (1967) Mechanisms of tachycardia caused by atropine in conscious dogs. *Am J Physiol* 212:901-910
11. Forssmann WG, Triepel J, Daffner C, Heym Ch (1988) Vasoactive intestinal peptide in the heart. *Ann NY Acad Sci* 527:405-420
12. Fujii AM, Vatner SF (1987) Baroreflex mechanisms buffering $\alpha$-adrenergic agonists in conscious dogs. *Am J Physiol* 253:H728-H736
13. Goldstein D (1983) Arterial baroreflex sensitivity, plasma catecholamines, and pressor responsiveness in essential hypertension. *Circulation* 68: 234-240
14. Heymans C, Neil E (1958) *Reflexogenic areas of the cardiovascular system.* London, Churchill
15. Heyndrickx GR, Boettcher DH, Vatner SF (1976) Effects of angiotensin, vasopressin, and methoxamine on cardiac function and blood flow distribution in conscious dogs. *Am J Physiol* 231:1579-1587
16. Hill MRS, Wallick DW, Martin PJ, Levy MN (1990) Excess tachycardia: an anesthetized preparation and its stimulation frequency response. *Proc Annu Int Conf IEEE Eng Med Biol Soc* 12:757-758
17. Hill MRS, Wallick DW, Martin PJ, Levy MN (1993) Frequency dependence of vasoactive intestinal polypeptide release and vagally induced tachycardia in the canine heart. *J Auton Nerv Syst* 43:117-122
18. Hodes R (1939) Exercise in the sympathectomized cat. *J Physiol* 126:171-179
19. Jourdan F, Morin G (1936) Mise en jeu réflexe des fibres accélératrices cardiaques du nerf pneumogastrique. *C R Soc Biol* 121:49-51
20. Kabat H (1940) The cardio-accelerator fibers in the vagus nerve of the dog. *Am J Physiol* 128: 246-257

21. Karasawa Y, Furukawa Y, Ren L-M, Takei M, Murakami M, Narita M, Chiba S (1990) Cardiac responses to VIP and VIP-ergic cholinergic interaction in isolated dog heart preparations. *Eur J Pharmacol* 187:9-17
22. Katona PG, Poitras JW, Barnett GO, Terry BS (1970) Cardiac vagal efferent activity and heart period in the carotid sinus reflex. *Am J Physiol* 218:1030-1037
23. Koizumi K, Kollai M (1992) Multiple modes of operation of cardiac autonomic control: development of the ideas from Canon and Brooks to the present. *J Auton Nerv Syst* 41:19-30
24. Lovell NH, Celler BG (1992) Investigation of post vagal tachycardias by analysis of the dynamics of cardiac period responses. *Proc Annu Int Conf IEEE Eng Med Biol Soc* 14:441-442
25. Machado BH, Brody MJ (1989) Contribution of neurogenic mechanisms to control of intrinsic heart rate. *Am J Physiol* 256:R231-R235
26. Rigel DF, Lipson D, Katona PG (1984) Excess tachycardia: heart rate after antimuscarinic agents in conscious dogs. *Am J Physiol* 246:H168-H173
27. Rigel DF (1988) Effects of neuropeptides on heart rate in dogs: comparison of VIP, PHI, NPY, CGRP, and NT. *Am J Physiol* 255:H311-H317
28. Robinson JL (1986) Effect of vasopressin and phenylephrine on arterial pressure and heart rate in conscious dogs. *Am J Physiol* 251:H253-H260
29. Roossien A, Brunsting JR, Nijmeijer A, Zaagsma J, Zijlstra WG (1992) Vagal cardioacceleration and sympathetic activity. *Proc 7th Int Catecholamine Symposium*, Amsterdam, congressbook p 275
30. Schuil HA (1987) *Vagale cardio-acceleratie en denervatie van het hondehart*. Thesis, University of Groningen, Van Denderen, Groningen, The Netherlands
31. Vatner SF, Higgins CB, Braunwald E (1974) Sympathetic and parasympathetic components of reflex tachycardia induced by hypotension in conscious dogs with and without heart failure. *Cardiovasc Res* 8:153-161
32. Versteeg PGA, Borgdorff P (1986) Influence of vagal cooling on cardiac output in normal and beta-blocked exercising dogs. *Eur J Appl Physiol* 54:617-623
33. Weihe E, Reinecke M, Forssmann WG (1984) Distribution of vasoactive intestinal polypeptide like immunoreactivity in the mammalian heart. *Cell Tissue Res* 236:527-540
34. Young MA, Hintze TH, Vatner SF (1985) Correlation between cardiac performance and plasma catecholamine levels in conscious dogs. *Am J Physiol* 248:H82-H88

# 5

# PERMANENT CANNULATION OF THE CANINE RIGHT CORONARY ARTERY


## Abstract

For long-term studies on the cardiovascular system, a stainless steel cannula has been developed, specially equipped for permanent implantation in the right coronary artery. By way of this cannula local injection of drugs into the blood supply of the sinoatrial node, measurement of coronary blood pressure, and sampling of coronary blood is made possible in conscious dogs. The cannula was successfully used for periods up to 15 months.


## Introduction

The arterial blood supply to the sinoatrial node in the dog is provided by a branch of the right coronary artery, the sinus node artery (6). In order to study the direct chronotropic properties of various drugs on the sinoatrial node in the absence of peripheral vascular effects, injection of small doses at or near the origin of the sinus node artery is necessary (4, 5, 8, 9). When this must be accomplished in long-term experiments in conscious dogs, a cannula should be implanted into the right coronary artery, just proximal to the origin of the sinus node artery.

Methods for permanent catheterization of coronary blood vessels in the dog were introduced by Herd and Barger (3), and have been used and modified by other investigators (2, 7, 12). These techniques consist of insertion of a Silastic catheter into the blood vessel via direct arteriotomy or side branch catheterization. However, several disadvantages are inherent in an indwelling catheter system in small blood vessels.

1. Placement of the catheter at exactly the right spot is difficult and disposition after correct insertion often occurs (4, 5). 2. Obstruction at the tip of the catheter may occur by endothelial overgrowth (12) or by formation of thrombi (3, 4, 5, 12). 3. Excessive angulation which easily occurs on insertion may block the Silastic catheter at the point of entry (4, 5, 12). 4. The indwelling tube impedes the normal sinus node artery blood flow

(4, 5, 12).

Therefore, we developed a type of cannula for implantation in the right coronary artery of the dog which obviates most of these disadvantages and which can be used for a long period. This chapter describes the construction of the cannula, the surgical procedure for implantation, and the regimen for maintenance and upkeep.

## Methods

### *Construction of the cannula*

The cannula, constructed from a stainless steel injection needle (Braun Melsungen AG; ID 0.009 in, OD 0.027 in), consists of three parts (Fig. 1). Part A is used to run the cannula through the wall into the lumen of the blood vessel, and out at the place of exit. Part B is the intraluminal part of the cannula. Part C makes the connection with the flexible catheter. D is the opening of the cannula which is cut in part B, E is the pointed tip of part A.

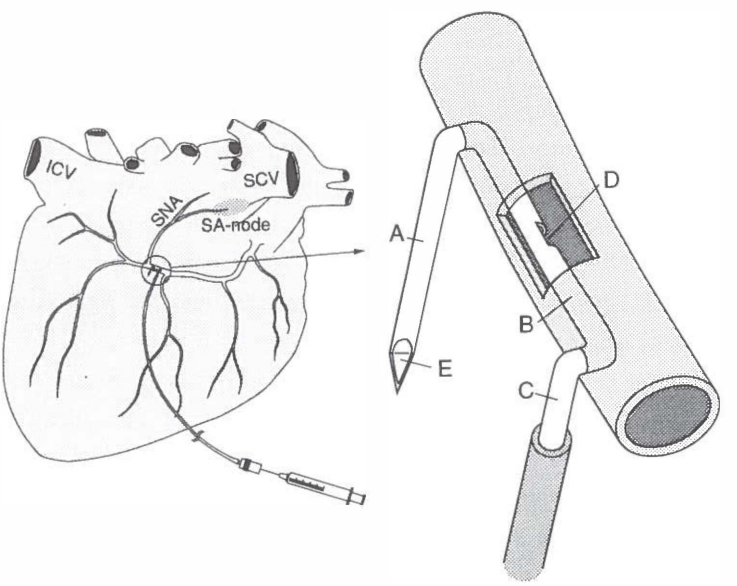



Fig. 1 Diagram of the cannula and its position in the right coronary artery. A and C are the legs of the cannula, B is the intraluminal part of the cannula, D is the hole in part B through which a drug can be injected into the coronary blood vessel, E is the pointed tip. ICV is the inferior caval vein, SNA is the sinus node artery, SCV is the superior caval vein, SA-node is sinoatrial node.

Construction of the cannula starts with insertion of a flexible wire into the stainless steel needle. This wire keeps the cannula patent during the subsequent procedure. Parts A and C are bent to put them at 90-degree angles with respect to part B; A and B are of equal length, to ensure that the place of exit of part A exactly fits the length of part B. Next, parts A and C are bent sidewards so that these parts become situated outside the plane of the central part B (Fig. 1). This provides the necessary leverage for fixing B to the inner wall of the blood vessel. The tip E of the cannula is then bent in the direction of C till it makes an angle of 45 degrees with the rest of part A, to avoid a stabwound in the posterior wall of the blood vessel during insertion. Finally, a hole (D) is cut in B (0.025 in). All surfaces are polished with emery paper. A flexible Silastic catheter (Tygon, S-54-HL; ID 0.020 in, OD 0.60 in) is attached to the terminal end of C by sliding it over a soldered rim made halfway on C. Sutures (Mersilene 3-0), on both sides of the rim, secure the connection. The other side of the catheter is closed with a stopper with injection port. The cannula/catheter system is sterilized by means of ethylene-oxide; prior to insertion it is filled with heparinized saline.

*Surgical procedure*

Ten adult mongrel dogs weighing 22 to 32 kg were first instrumented with an indwelling catheter in the pulmonary artery, two indwelling catheters in the superior caval vein, and one in the descending aorta, for fluid infusion, taking blood samples, and blood pressure measurement during the experiments. The catheters in the pulmonary artery and superior caval vein were introduced through the omocervical vein. The aortic catheter was introduced through the omocervical artery. The catheters were tunnelled subcutaneously and brought out in the midscapular region. The exterior parts of the catheters were protected by putting them in a pocket of the jacket, continuously worn by the dogs after the first instrumentation.

After at least 7 days, the coronary cannula was implanted by means of the following procedure. Neuroleptanesthesia was induced by fentanyl (0.1 mg/kg, i.m.) and fluanisone (5 mg/kg, i.m.), and analgesia maintained by fentanyl infusion (0.3 mg/h, i.v.). Spontaneous ventilation was suppressed by means of alcuronium (3 mg and 0.6 mg/h, i.v.). After endotracheal intubation the dogs were ventilated mechanically with a mixture of $N_2O$ and $O_2$ in a ratio of 2:1, using intermittent positive pressure (Pulmomat 19K1). Minute ventilation was adjusted to maintain end-tidal $pCO_2$ (Capnograph) within 4.0-4.7 kPa. Blood samples were periodically taken from the catheter in the aorta for the determination of $pO_2$, $pCO_2$, pH (Radiometer ABL2) and oxygen saturation (Radiometer OSM2). If necessary, sodium bicarbonate (500 mmol/l) was given intravenously to

maintain the acid-base state within the normal range. Body temperature was determined with an intramuscular thermocouple (Ellab TE3), and was maintained in the range of 37.5-38.5 °C by means of a thermostatically controlled heating pad. Needle electrodes were attached to the legs for recording a lead II electrocardiogram. During the testing of different drugs on their chronotropic action, the heart rate was obtained from the ECG by means of a cardiotachometer (ECG module, Philips V 692). Systemic arterial blood pressure was measured with a strain-gauge manometer (Statham P23Db) through the implanted aortic catheter. The signals were amplified, analog-to-digital converted, and fed into a computer for data processing and display. At the time of surgery, $\beta$-adrenergic receptor blockade was achieved by means of timolol (5 mg, i.v.) in order to reduce the heart rate.

A right anterolateral thoracotomy was performed in the fourth intercostal space. The pericardium was incised parallel to the phrenic nerve and the right auricle was lifted out of the pericardial sac exposing the coronary groove, and retaining sutures were placed. Proximal to the origin of the sinus node artery, the anterior wall of the right coronary artery was exposed over about 15 mm by careful dissection. Without occlusion of the artery, a purse-string suture consisting of 5 fine stitches (Novafil, 6-0, non-resorbable) was placed in the anterior vessel wall after defining the right place of entry, just proximal to the origin of the sinus node artery. After each stitch, bleeding was stopped by applying light pressure to the area with a wet cotton wool stick. Using a curved atraumatic artery forceps covered with Silastic tubing, the cannula was inserted through the stabwound made by its tip, inside the purse-string suture. As soon as the pointed tip (E) had penetrated the wall of the blood vessel, part A was brought in the longitudinal direction of the artery, to avoid a stabwound in the posterior wall of the blood vessel. Then, the total length of part A was pushed gently in the direction of blood flow; in this way the exit place of the tip (E) was defined. The pointed tip was then run outwards through the vessel wall, and part A was pulled out of the blood vessel till part B had reached its intraluminal position. Part A of the cannula was then covered and closed by a heat-sealed polyethylene cap. A purse-string suture (Novafil, 6-0), surrounding part A, was placed in the artery wall. The legs of the cannula (A and C), were sutured to the surface of the right ventricle with non-obstructive looping ligatures (Dexon Plus, 3-0), which were drawn tautly but not too tightly. These ligatures help to avoid overturn of the cannula. Finally, the purse-string sutures were tautened, to stop any leakage of blood.

The intraluminal position of the cannula was ascertained by observing backflow of blood into the catheter. The catheter attached to the cannula was placed kink-free inside the pericardial sac and brought out through a separate hole, and fixed with a purse-

string suture (Dexon Plus, 3-0). Then, the auricle was freed and the pericardium was closed by continuous suturing (Catgut, 2-0). After tunnelling subcutaneously, the catheter was brought out in the midscapular region, to join the previously implanted catheters. The chest was closed in layers, negative intrapleural pressure established, and the lungs inflated to maximum volume for a few seconds. The dogs were allowed to recover from surgery for at least 14 days before the experiments were performed.

*Pre- and postoperative care; upkeep of the cannula*

Two days before and five days after surgery the dogs were put on a daily antibiotic regimen (0.5 g penicillin G, 0.5 g streptomycin, i.m.), and treated with dipyridamole (75 mg) and aspirin (200 mg), given orally. After surgery, the dogs were given buprenorphine (0.5 mg, i.m.) for at least two days. The dogs wore jackets to protect the catheters from being chewed on. The exterior parts of the catheters were protected by putting them in a pocket of the jacket containing a gauze soaked in disinfectant (chloorhexidine). Patency and sterility of the cannula-catheter system was maintained by daily flushing with an NaCl solution (154 mmol/l), and subsequently filled with a mixture of heparin (5.000 U/ml, LEO), penicillin G (20.000 U/ml) and saline.

**Results**

Of the ten dogs used, one was terminated after two months by means of an overdose of pentobarbital, because a crack had occurred in the cannula at the site of the hole D. In one other dog the cannula could not be fixed properly because of the absence of the pericardium and soon became dislocated; this dog was terminated after about one month. The other eight dogs remained in good health and were subjected to several experiments (10). They were terminated by the injection of an overdose of pentobarbital between 7 and 15 months after the implantation.

Due to its shape, it was in all cases possible to bring the cannula exactly in the intended position. Bleeding at the entry and exit holes could immediately be stopped after closing the purse-string sutures. Clots which occasionally developed in the cannula were successfully removed by intracoronary infusion of nitroglycerin (0.02 mg/min, during 20 min), followed by streptokinase (1.500 U/min, during 60 min).

Proper functioning of the cannula and the functional integrity of the cannulated blood vessels were ascertained by means of coronary arteriography, and ECG and blood pressure recordings. Fig. 2 shows a coronary arteriogram obtained seven months after implantation of the cannula. Contrast material injected into the coronary cannula

demonstrates that the cannula lies proximal to the origin of the sinus node artery. There is no evidence of trauma, distortion or thrombus formation. A lead II ECG and recordings of aortic and coronary arterial blood pressure, made on the same day as the coronary arteriogram, are shown in Fig. 3. The ECG shows a normal sinus rhythm. The coronary arterial pressure curve is compatible with normal perfusion of the vessel. An injection of a small dose of acetylcholine (10 $\mu$g in 0.5 ml saline) into the coronary artery proved to be an useful procedure to check whether the cannula was patent. This consistently produced a sinus bradycardia or sinus arrest. Recovery of the sinus rhythm following the acetylcholine-induced bradycardia was always prompt and sustained (Fig. 4).

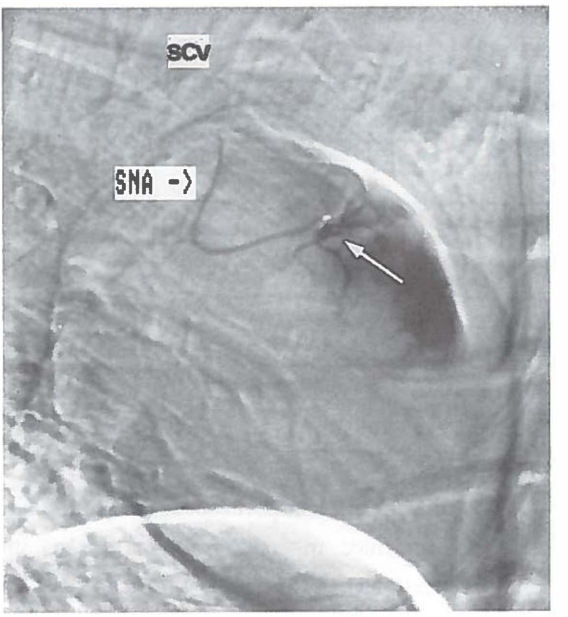



Fig. 2 Arteriogram made seven months after implantation of the cannula. SCV is the superior caval vein, SNA is the sinus node artery. The arrow shows the place of the injection of contrast material in the right coronary artery, through the hole of the cannula.


Fig. 3 ECG and blood pressure tracings. From top to bottom: time (s), ECG (lead II), arterial blood pressure (Pao) (mmHg), blood pressure in the right coronary artery (Pcor) (mmHg). There is a normal sinus rhythm with a respiratory arrhythmia, as usually present in a healthy dog at rest.

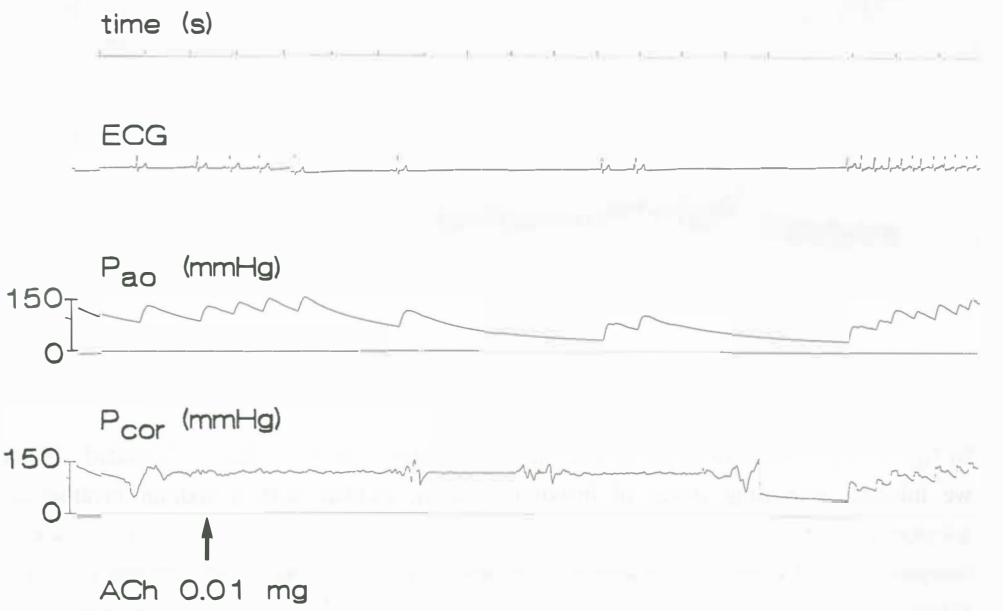



Fig. 4 Effects of acetylcholine (ACh). From top to bottom: time (s), ECG (lead II), arterial blood pressure (Pao) (mmHg), blood pressure in coronary artery (Pcor) (mmHg). Note that during the injection of acetylcholine the recording of the coronary blood pressure is not possible.

The cannula-catheter system was used to identify the terminal transmitter in the vagal cardiac accelerator system. We studied the chronotropic effects of acetylcholine, histamine and vasoactive intestinal polypeptide (VIP) in $\beta$-adrenoceptor and muscarinic receptors blocked conscious dogs, by injecting these substances into the right coronary artery. A low dose of acetylcholine (0.01 mg) (fig. 5) injected into $\beta$-adrenoceptor blocked dogs resulted in cardiac arrest for several seconds, without disturbing the dog's behavior. After additional muscarinic receptor blockade, acetylcholine in doses up to 0.5 mg did not influence the heart rate, *i.e.* the prevailing vagal cardiac acceleration, which not only confirms the adequacy of the muscarinic receptor blockade (methylatropine 0.5 mg/kg and 0.5 mg/kg/h), but also excludes the involvement of cardiac nicotinic receptors in the VCA pathway.

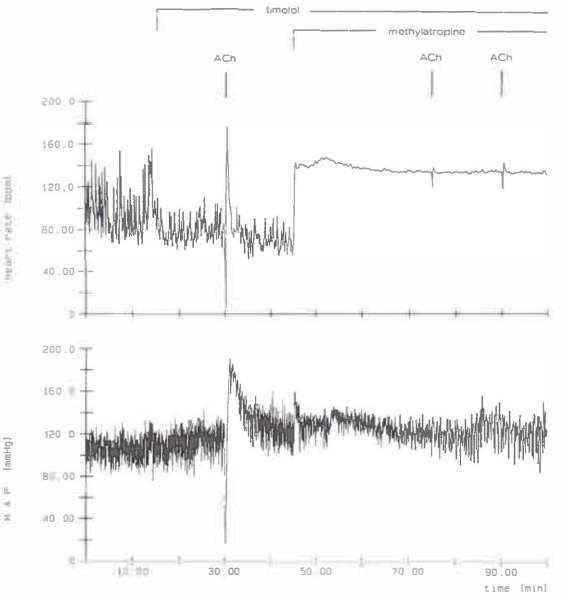



Fig. 5 Heart rate (beats/min) and mean arterial blood pressure (MAP; mmHg) responses to acetylcholine injected intracoronary *before* (0.01 mg; left) and *after* muscarinic receptor blockade (0.1 and 0.5 mg; right) in a $\beta$-adrenoceptor blocked conscious dog.

In fig. 6 and 7 the usefulness of the cannula-catheter system is clearly illustrated. When we injected increasing doses of histamine (Bufa), diluted with a sodium bicarbonate solution (167 mmol/l), into the right coronary artery of $\beta$-adrenoceptor and muscarinic receptor-blocked dogs only moderate increases in heart rate were encountered (Fig. 6). When the dose of histamine was increased, the heart rate increase remained moderate and was accompanied by a decrease in arterial blood pressure. Furthermore, the heart rate increase to higher doses of histamine became biphasic, which probably resulted from the

combination of a direct positive chronotropic effect of histamine and reflex decrease in vagal cardiac acceleration induced by the fall in arterial blood pressure. This makes histamine an improbable candidate for the terminal transmitter in the vagal cardiac accelerator pathway. In fig. 7 it is shown that when VIP (Sigma) is injected intravenously a pronounced increase in heart rate is accompanied by a marked decrease in mean arterial blood pressure.

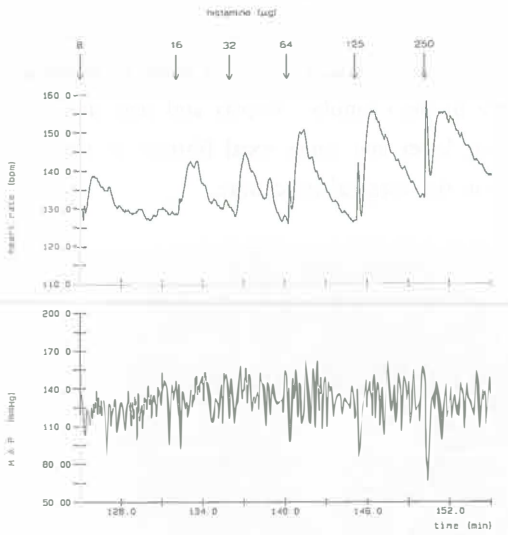



Fig. 6 Heart rate (beats/min) and mean arterial blood pressure (MAP; mmHg) responses to an increasing dose of histamine (8, 16, 32, 64, 125 and 250 $\mu$g) injected intracoronary into a $\beta$-adrenoceptor and muscarinic receptor blocked conscious dog. Note that a small dose of histamine produces a monophasic increase in HR, while a large dose of histamine causes a biphasic increase in HR which is accompanied by a fall in MAP.

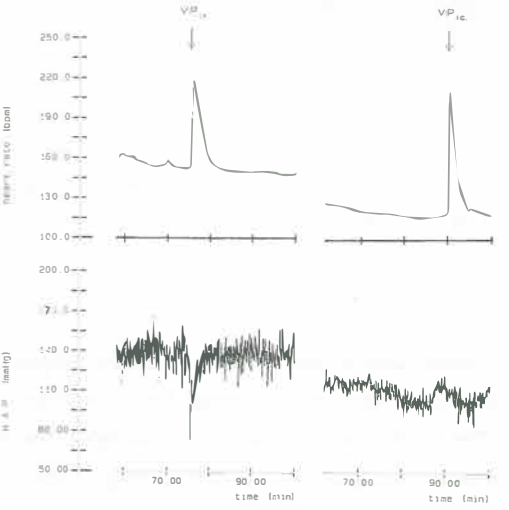



Fig. 7 Heart rate (beats/min) and mean arterial blood pressure (MAP; mmHg) responses to vasoactive intestinal polypeptide (VIP) injected intravenously (i.v.) (10 $\mu$g; left panel) and intracoronary (i.c.) (0.5 $\mu$g; right panel) in a $\beta$-adrenoceptor and muscarinic receptor blocked conscious dog. Note that the intracoronary injection of a low dose of VIP causes a pronounced increase in HR without causing a major change in MAP.

Some authors suggested that the tachycardia is not produced by VIP but that the increase in heart rate emanated from the VIP-induced decrease in blood pressure (1, 11) With the use of the cannula-device we were able to demonstrate the positive chronotropic property of VIP; a small amount of intracoronary injected VIP resulted in an increase in heart rate similar to that seen after intravenous injection but without causing a fall in mean arterial blood pressure.

Autopsy and histological examination of the coronary blood vessel revealed no sign of obstruction or thrombus formation. Fig. 8 shows the right coronary artery at the site of cannula implantation with a patent lumen. The cannula was removed prior to preparation for microscopy. At the entry and exit place of the cannula, fibrosis and scar tissue were found with interruption of the internal elastic layer and some focal fibrosis of the medial layer of the vessel wall. The latter was due to the surgical procedure.

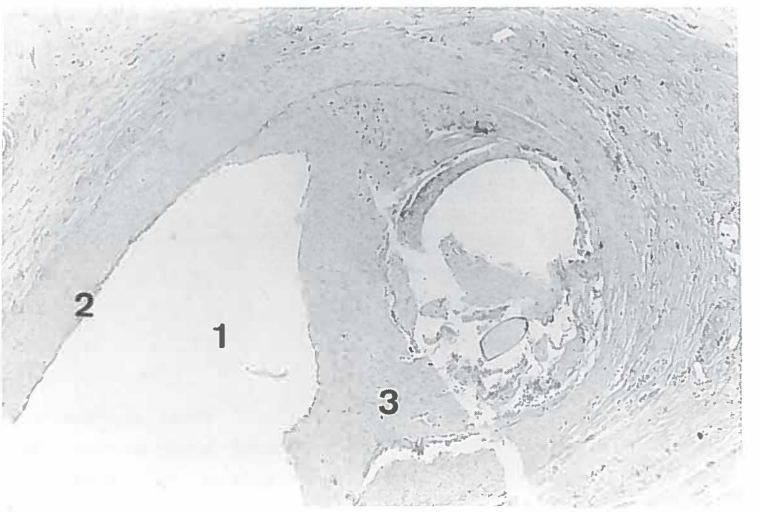



Fig. 8 Histological examination of the cannulated vessel after autopsy. Intact lumen of the right coronary artery (1). Internal elastic layer (2). Fibrose tissue at the site of implantation (3).

## Discussion

We demonstrated that a cannula-catheter system can be constructed for implantation in the right coronary artery of the dog, which remains functional for a long period, without impairing coronary blood flow. The system was shown to be suitable for the measurement of coronary arterial blood pressure, and for the injection of drugs (10). The direct action of a drug on the sino-atrial node can be established through the injection of a

minute amount, which does not give rise to a pharmacologically active concentration in the pulmonary or the systemic circulation, as is shown for histamine and vasoactive intestinal polypeptide (10).

Our cannula design allows a less complicated implantation procedure than the catheters used by others: bonding of pieces of tubing at the time of surgery (3), treating the surface of the blood vessel with formaldehyde (7), and stripping the adventitia (2, 7) are not necessary. The special shape of the cannula guarantees a proper and reliable vessel fit. In contrast with previously described methods (4, 5) our cannula can be precisely brought into its position and once properly fixed in the artery, it does not become dislocated. A small work field appears to be adequate for insertion of the cannula. Elimination of side branches, as performed in the procedures of James and Nadeau (4, 5), and Rigel (9), is not necessary, which diminishes the risk of impairing the coronary perfusion. Because of its construction, and the chosen material, the intravascular foreign body is small (4 mm). As a consequence, thrombus formation and embolization hardly occurs. The frequency of flushing (twice a day), guarantees a permanent passage notwithstanding the small amount of fibrose tissue, which is present at the entry and exit places of the cannula, and thus keeps the vessel patent.

Our type of cannula can also be used for implantation in other blood vessels. It may be particularly suitable for implantation at difficultly accessible sites, such as renal arteries and veins. The cannula can, besides for blood pressure measurement and drug injection, be used for blood sampling.

## References


1. Domschke S, Domschke W (1982) Biological actions of vasoactive intestinal peptide in man. In: Said SI (Ed) *Vasoactive Intestinal Peptide.* Raven Press, New York, p. 201-209
2. Gwirtz PA (1986) Construction and evaluation of a coronary catheter for chronic implantation in dogs. *J Appl Physiol* 60:720-726
3. Herd JA, Barger AC (1964) Simplified technique for chronic catheterization of blood vessels. *J Appl Physiol* 19:791-792
4. James TN, Nadeau RA (1962) Direct perfusion of the sinus node: an experimental model for pharmacologic and electrophysiologic studies of the heart. *Henry Ford Hosp Med Bull* 10:21-25
5. James TN, Nadeau RA (1963) Sinus bradycardia during injections directly into the sinus node artery. *Am J Physiol* 204:9-15
6. Meek WJ, Keenan M, Theisen HJ (1929) The auricular blood supply in the dog. *Am Heart J* 4:591-598
7. Rankin JS, McHale PA, Greenfield Jr JC (1976) Nonobstructive chronic cannulation of the left circumflex coronary artery of the dog. *J Appl Physiol* 40:263-265
8. Rigel DF, Katona PG (1986) Effects of antihistamines and local anesthetics on excess tachycardia

in conscious dogs. *J Pharmacol Exp Ther* 238:367-371

9. Rigel DF (1988) Effects of neuropeptides on heart rate in dogs: comparison of VIP, PHI, NPY, CGRP, and NT. *Am J Physiol* 255:H311-H317
10. Roossien A, Brunsting JR, Zaagsma J, Zijlstra WG (1990) Vasoactive intestinal polypeptide: a probable mediator of vagal cardioacceleration in dogs. *Eur J Physiol* 420:R88
11. Smitherman TC, Dehmer GJ, Said SI (1988) Vasoactive intestinal peptide as a coronary vasodilator. *Ann NY Acad Sci* 527:421-430
12. Young SH, Stone HL (1974) A new indwelling catheter for coronary sinus blood sampling in dogs. *J Appl Physiol* 36:767-768

# 6

# VASOACTIVE INTESTINAL POLYPEPTIDE: THE PROBABLE MEDIATOR IN THE VAGAL CARDIAC ACCELERATOR SYSTEM IN DOGS


**Abstract**

The vagal cardiac accelerator (VCA) system takes part in the autonomic nervous control of the heart rate (HR). In the present study we tried to adduce evidence for vasoactive intestinal polypeptide (VIP) as the terminal transmitter in the VCA pathway. The effect of VIP on HR and mean arterial blood pressure (MAP) was investigated after unmasking the VCA activity by blocking the sympathetic accelerator and vagal decelerator influences on HR in permanently instrumented conscious dogs. Following intravenously administered VIP (10 $\mu$g) HR increased by 43$\pm$7 to 209$\pm$7 beats/min and MAP decreased by 48$\pm$3 to 79$\pm$5 mmHg. After VCA activity was reflexly enhanced (38$\pm$7 beats/min), following $\alpha_1$-adrenoceptor stimulation, exogenous VIP increased HR by 37$\pm$7 to 217$\pm$6 beats/min and decreased MAP by 79$\pm$6 to 89$\pm$5 mmHg; hence the VIP-induced tachycardia is attenuated when vagal nerve activity is increased within the same experiment. Similarly, the increase in HR to exogenous VIP is inversely related to the prevailing VCA activity, as established in the different experiments. The VIP tachycardia is due to a direct effect of VIP on the pacemaker activity and is certainly not reflexly induced by the fall in MAP, because intracoronary administered VIP (0.5 $\mu$g) caused an increase in HR by 64$\pm$13 to 207$\pm$12 beats/min which was not accompanied by a major change in MAP (-8$\pm$2 mmHg). In addition, intravenously administered VIP (10 $\mu$g) caused also a tachycardia in vagotomized dogs with blocked $\beta$-adrenoceptors. The administration of the VIP antagonists [D-p-Cl-Phe$^6$,Leu$^{17}$]-VIP (50-150 $\mu$g, i.c.) and [Lys$^1$,Pro$^{2,5}$,Leu$^{17}$]-VIP (20 $\mu$g, i.c.) did not result in alterations in VCA activity nor did the VIP antagonists block the VCA reflex response to a rise in MAP. Furthermore, neither VIP antagonist did reduce the VIP tachycardia. By means of a cannula specially developed to inject substances directly into the blood supply of the sinus node in conscious dogs, the powerful positive chronotropic effect of VIP has been established in intact $\beta$-adrenoceptor and muscarinic receptor blocked conscious dogs. The inverse

relation between the VIP tachycardia and the prevailing VCA activity suggests that VIP is involved in the transmission of the VCA impulse. Conclusive evidence, though, has to wait for the availability of a specific cardiac VIP receptor antagonist.

## Introduction

The theory that the chronotropic control of the heart by the autonomic nervous system is exerted through acetylcholine and norepinephrine, released by the vagal decelerator and sympathetic accelerator system, is incomplete. It has long been established that a vagal cardiac accelerator (VCA) system acts in concert with the classical systems (4, 5, 11, 22), although the VCA had to be rediscovered several times before it became more widely accepted (7, 12, 20, 28).

The transmitter released by the VCA system has not yet been identified, but since its activity is unmasked by combined adrenergic and muscarinic receptor blockade, it is definitely of a non-adrenergic, non-cholinergic type. Recently, evidence has been presented supporting the hypothesis that neuropeptides, including VIP, secretin, glucagon, neurotensin, neuropeptide Y, substance P and calcitonin gene-related peptide, contribute to cardiac control(10, 29, 35). Of these neuropeptides VIP has been claimed as a probable transmitter in the VCA system, because VIP containing neurons (1, 41) and VIP receptors (9, 19) have been found in the mammalian heart. Since the distribution of VIP-immunoreactive neurons resembles that of postganglionic parasympathetic neurons, VIP has been associated with vagal nerves (41). The VIP-immunoreactivity may also originate from intrinsic cardiac neurons as suggested by Anderson and coworkers (1). VIP has been proposed to produce VCA activity, because of its powerful positive chronotropic effect (29, 31, 33) and because similar high frequencies of electric vagal nerve stimulation are required to activate the VCA system and to release endogenous VIP (2, 18, 20). Moreover, VCA activity and VIP release are both suppressed by nicotinic receptor blockade (7, 12, 13).

Nonetheless, the relationship between VCA system and VIP has only been investigated by comparing the VIP tachycardia with the stimulation-induced VCA activity (18, 29, 40) or by studying the effect of VIP antagonist [D-p-Cl-Phe$^6$, Leu$^{17}$]-VIP on the stimulation-induced VCA activity (18, 40). In the latter studies, VIP was suggested to be the mediator of VCA activity because VIP antagonist [D-p-Cl-Phe$^6$, Leu$^{17}$]-VIP reduced (18, 40) the stimulation-induced VCA activity. However, the relation between the *inherent* VCA activity and VIP has never been analyzed. This relation can only be investigated in conscious dogs, because during the use of anesthesia inherent VCA activity

is suppressed by the vagolytic effect of anesthetics (7, 28). Furthermore, VIP needs to be injected directly into the sinus node artery, because intravenous administration of VIP results, besides in a tachycardia, in a marked decrease in arterial blood pressure which may reflexly alter the heart rate response to VIP. Because of this decrease in blood pressure some authors even suggested that the tachycardia following VIP is entirely reflexly induced and that VIP is devoid of a chronotropic effect (24, 36).

In the present study, the chronotropic property of VIP and the potential role of VIP in the VCA system was investigated by studying the VIP tachycardia superimposed on the inherent VCA activity in conscious dogs. Some of them were chronically equipped with a cannula in the right coronary artery to allow injection of VIP directly into the sinus node artery in order to circumvent baroreceptor reflex mediated changes in heart rate. In addition, the effects of the VIP antagonists [D-p-Cl-Phe$^{6}$, Leu$^{17}$]-VIP and [Lys$^{1}$, Pro$^{2,5}$, Leu$^{17}$]-VIP on the VCA activity, on the VCA reflex response to a blood pressure rise, and on the VIP-induced tachycardia were examined.

## Methods

*Surgical preparations*

The experiments were performed on adult mongrel dogs of both sexes (26-43 kg). Indwelling catheters (Tygon microbore S-54-HL, ID 0.5 mm, OD 1.5 mm) were inserted into the right omocervical vein and artery. The tips were advanced to the superior caval vein and descending aorta, and the proximal ends tunneled subcutaneously and secured to the skin in the midscapular region. To allow injection of drugs directly into the sinus node artery ($n$=8), we chronically cannulated the right coronary artery according to a recently developed technique of Nijmeijer, Roossien and Brunsting (26). A cannula, constructed of a stainless steel injection needle (Sterican 0.55X25 mm, Braun Melsungen), was implanted proximal to the origin of the sinus node artery. A flexible catheter, connected to the cannula, was tunneled subcutaneously and secured to the skin in the midscapular region. The coronary cannula had a dead space of 0.3 ml. The surgical procedures were carried out under neuroleptanesthesia, using fentanyl (0.1 mg/kg, i.m. and 0.01 mg/kg/h, i.v.) and fluanisone (5 mg/kg, i.m.) (Hypnorm, Janssen), supplemented with a mixture of $N_2O$ and $O_2$ (ratio 2:1). The catheters were kept patent by flushing with an NaCl solution (154 mmol/l) and filling with heparinized saline once (venous and arterial catheter, 2.000 U/ml) or twice a day (coronary cannula, 5.000 U/ml). The dogs wore jackets to protect the catheters from being chewed on. The first experiment was carried out after at least 14 days. The study protocol was approved by the

appropriate Animal Care and Use Committee (Groningen University, FDC 65-0186).

*Experimental protocols*

Before the experiments were performed, the dogs were habituated to the laboratory surroundings and personnel, and trained to stand quietly on a padded table in a modified Pavlovian sling. To minimize external influences that might excite the animals, the room was dimly lit and the sound level kept as low as possible. In each experiment the prevailing VCA activity was unmasked through blocking $\beta$-adrenergic and muscarinic receptors, 15 min and 45 min after the start of the experiment, respectively. The experiments were separated by an interval of at least 14-days. In protocol I the influence of intravenously administered VIP (10 $\mu$g) on HR and MAP was examined 30 min after the muscarinic receptor blockade. Subsequently, MAP was elevated, via $\alpha_1$-adrenoceptor stimulation, to induce an increase in VCA activity. Then, 15 min later, VIP (10 $\mu$g) was injected a second time. To study the direct chronotropic response to VIP on the pacemaker cells, two additional experiments were carried out in which VIP (10 $\mu$g) was intravenously administered into vagotomized dogs with blocked $\beta$-adrenoceptors and muscarinic receptors; bilateral cervical vagotomy was performed on the day before these experiments (protocol II). In addition, the response to an increase and to a decrease in MAP, generated through the $\alpha_1$-adrenoceptor agonist methoxamine (0.1 mg/kg, i.v.) and antagonist doxazosin (0.1 mg/kg, i.v.), respectively, was studied in these vagotomized animals.

To preclude any reflex effects due to the VIP-induced fall in MAP, VIP was injected directly into the right coronary artery in protocols III, IV and V. In protocol III we examined the chronotropic effect of increasing doses of VIP (0.05, 0.1, 0.25, 0.5, 1, 1.5, 2 and 3 $\mu$g) administered in equal volumes (1.5 ml). Between the VIP injections sufficient time was allowed for the circulatory parameters to return to their former level. In protocol IV VIP (0.5 $\mu$g) was injected before and after reflexly increasing the VCA activity through $\alpha_1$-adrenoceptor stimulation. To verify that the tachycardia was indeed VIP-induced and not due to the solvent or the intracoronary injection procedure, an equal volume of solvent was injected intracoronarily. In protocol V the influence was examined of two VIP receptor antagonists, [Lys$^1$, Pro$^{2,5}$, Leu$^{17}$]-VIP (20 $\mu$g, i.c.) and [D-p-Cl-Phe$^6$, Leu$^{17}$]-VIP (50-150 $\mu$g, i.c.), on the VCA activity and on the VCA reflex response to a rise in MAP. In addition, the potency of the VIP antagonists to inhibit the VIP-induced tachycardia was tested by injecting the VIP agonist (0.5-5 $\mu$g, i.c.) before and after the administration of the VIP antagonist.

*Circulatory measurements*

A lead II electrocardiogram was recorded using disposable Ag/AgCl electrodes. HR was determined from the ECG with a cardiotachometer (Philips V 692). Systemic arterial blood pressure was measured through the implanted catheter in the aorta and measured with a strain-gauge manometer (Uniflow, Baxter). The signals were amplified, analog-to-digital converted, and fed into a computer for data processing and display.

*Drugs*

The $\beta$-adrenergic receptors were blocked with timolol (0.2 mg/kg and 0.2 mg/kg/h) (Sigma). Methylatropine (0.5 mg/kg and 0.5 mg/kg/h) (Sigma) was used as an antimuscarinic agent. The $\alpha_1$-adrenergic receptor agonist methoxamine (0.1 mg/kg) (Wellcome) and doxazosin (0.1 mg/kg) (Pfizer) were administered to increase and decrease MAP, respectively. All drugs were dissolved in an NaCl solution (154 mmol/l) and administered intravenously. Porcine-VIP agonist (Sigma) and the VIP antagonists, [D-p-Cl-Phe$^6$, Leu$^{17}$]-VIP (Sigma) and [Lys$^1$, Pro$^{2,5}$, Leu$^{17}$]-VIP (Sigma), dissolved in acetic acid (50 mmol/l) were diluted with a sodium bicarbonate solution (167 mmol/l).

*Data analysis*

The activity of the VCA system is educed through blocking $\beta$-adrenoceptors and muscarinic receptors. Its activity is quantified by subtracting the denervated HR from HR determined after combined $\beta$-adrenoceptor and muscarinic receptor blockade. The results are expressed as means $\pm$ S.E.M. Results were analyzed using the Student's paired *t*-test. Correlations were calculated using Pearson correlation coefficient. A $P$ value $\leq 0.05$ was considered significant.

**Results**

As shown in a representative protocol I experiment in one dog (Fig. 1), intravenous VIP injection resulted in a pronounced tachycardia and a marked decrease in MAP. When in $\beta$-adrenoceptor blocked dogs in protocol I the muscarinic receptors were blocked, HR increased to a maximum level of $182\pm10$ beats/min and declined to $166\pm8$ beats/min in 30 min. Following the subsequent administration of VIP, HR increased by $43\pm7$ beats/min and MAP simultaneously decreased by $48\pm3$ mmHg (Fig. 2*A*).

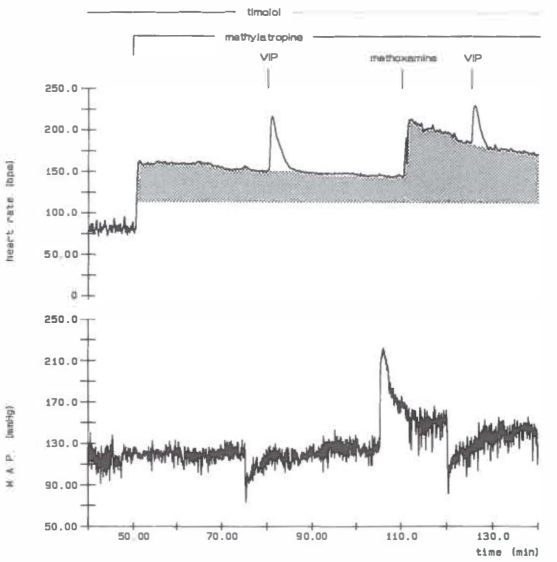



Fig. 1 Representative protocol I experiment: responses in heart rate (beats/min) and mean arterial blood pressure (MAP; mmHg) to $\beta$-adrenoceptor and muscarinic receptor blockade and to subsequent administrations of VIP (10 $\mu$g i.v.), methoxamine (0.1 mg/kg, i.v.) and VIP (10 $\mu$g, i.v.) in a conscious dog. The VCA activity is represented by the shaded area. Note that when MAP is increased using methoxamine, HR increases. In both instances, VIP elicits a transient increase in HR and decrease in MAP.

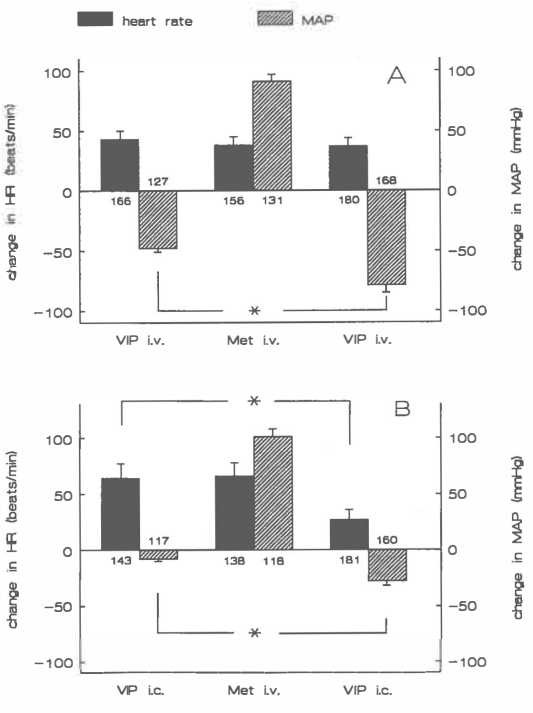



Fig. 2 Responses in heart rate (beats/min) and mean arterial blood pressure (MAP; mmHg) to VIP, injected before and after stimulation of $\alpha_1$-adrenoceptors by methoxamine (Met; 0.1 mg/kg, i.v.) in $\beta$-adrenoceptor and muscarinic receptor blocked dogs. HR and MAP values prior to the injection of the drugs are reported below or above the bars that represent the response to the intervention. Panel A shows an increase in HR and a fall in MAP to intravenously administered VIP (10 $\mu$g) ($n$=11). When MAP was elevated using methoxamine, an increase in HR was observed. Subsequent administration of VIP resulted in an increase in HR and a significantly larger fall in MAP (protocol I). Panel B shows that intracoronary VIP injection (0.5 $\mu$g) elicited an increase in HR and a small decrease in MAP ($n$=6). After elevating MAP, VIP elicited an attenuated increase in HR and an enhanced, but still moderate, decrease in MAP (protocol IV). An asterisk denotes a significant difference.

The increase in HR was inversely related to HR prior to VIP ($n$=11, r=-0.591, $P$=0.05). After stimulating $\alpha$1-adrenoceptors, MAP increased by 91±6 mmHg and HR increased by 38±7 beats/min to a maximum of 194±7 beats/min. The second VIP injection produced an attenuated acceleration of 37±7 beats/min and a significantly potentiated fall in MAP of 79±6 mmHg. The increase in HR was again inversely related to HR prior to VIP ($n$=11, r=-0.615, $P$=0.04). The HR and MAP responses to $\alpha_1$-adrenoceptor stimulation were long-lasting, whereas the responses to VIP were transient. In some animals, which showed a high HR following muscarinic receptor blockade (*i.e.* high VCA activity), VIP resulted in a moderate increase in HR which was followed by a decrease before returning to its previous level (Fig. 3).

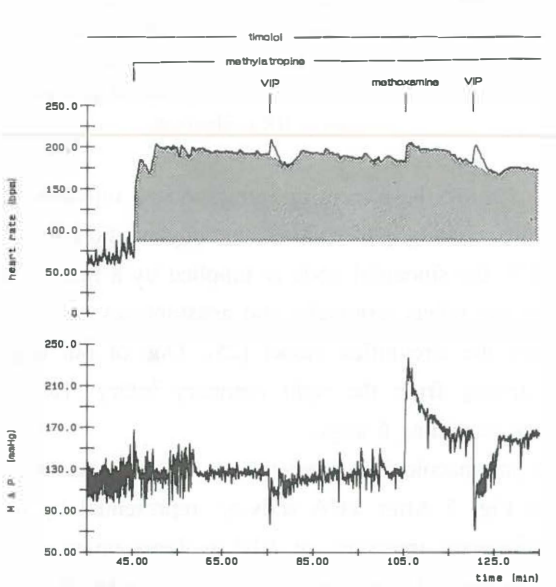



Fig. 3 Protocol I experiment in a dog with a high VCA activity. Responses in heart rate (beats/min) and mean arterial blood pressure (MAP; mmHg) to $\beta$-adrenoceptor and muscarinic receptor blockade and to subsequent administrations of VIP (10 $\mu$g, i.v.), methoxamine (0.1 mg/kg, i.v.) and VIP (10 $\mu$g, i.v.). The VCA activity is represented by the shaded area. The denervated HR (84 beats/min) was determined in a separate experiment following $\beta$-adrenergic and muscarinic receptor blockade after vagotomy had been performed on the day before that experiment. VIP elicited only moderate increases in HR which are accompanied by large decreases in MAP. The VIP-induced tachycardias are followed by a brief decrease in HR.

When in the two vagotomized dogs the $\beta$-adrenergic and muscarinic receptors were blocked, HR stabilized at a level of 89 and 109 beats/min, which may be considered to be their denervated HR. In the preceding protocol I experiment on these dogs, HR increased to 208 and 218 beats/min following muscarinic receptor blockade. The VCA activity thus amounted to a maximum of 208-89=119 and 218-109=109 beats/min, respectively. When VIP was injected in the vagotomized state, HR increased by 50 beats/min (Fig. 4) and 31 beats/min, respectively. When MAP was decreased or increased, through doxazosin and methoxamine, respectively, no appreciable changes in HR ($\leq$5 beats/min) occurred in the vagotomized animals (Fig. 4).

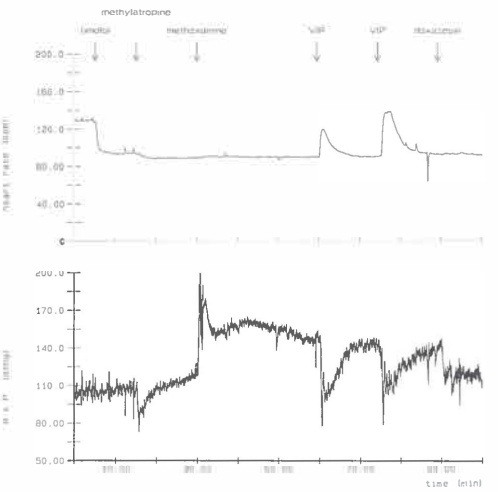



Fig. 4 Protocol II experiment: heart rate (beats/min) and mean arterial blood pressure (MAP; mmHg) responses to methoxamine (0.1 mg/kg, i.v.), VIP (5 and 10 $\mu$g, i.v.) and doxazosin (0.1 mg/kg, i.v.) in a $\beta$-adrenoceptor and muscarinic receptor blockade conscious dog which had its vagal nerves transected the day before the experiment. Note that after $\beta$-adrenoceptor and muscarinic receptor blockade HR stabilized at a level of 89 beats/min, *i.e.* the denervated HR, and that the administration of methoxamine and doxazosin nor their resulting changes in MAP induced major changes in HR, while following the VIP injection a pronounced increase in HR is observed.

Initially, eight dogs were used to study the effects of intracoronary injection of VIP. Of these dogs, one died during the recovery period after the implantation of the cannula by a pneumothorax. Usually (85%) the sinoatrial node is supplied by a branch of the right coronary artery which traverses the sulcus terminalis and anastomoses with one or more atrial branches which arise from the circumflex vessel (25). One of our dogs appeared to lack a sinus node artery arising from the right coronary artery. Hence, further experiments were conducted on the remaining 6 dogs.

The HR and MAP responses to pharmacological interventions in a representative protocol III experiment are displayed in Fig. 5 After VCA activity, represented by the shaded area, has been uncovered, intracoronary injections of VIP in doses up to 3 $\mu$g induced increases in HR which were accompanied by only minor alterations in MAP.

Fig. 6 shows the HR and MAP responses to increasing doses of VIP, as established in 4 protocol III experiments. As shown in Fig. 1 intravenous injection of VIP resulted in a transient increase in HR that is similar to that found when VIP was injected intracoronarily (Fig. 5). In Fig. 7 a protocol IV experiment is shown in which intracoronary injection of VIP elicited a pronounced tachycardia which was not accompanied by a decrease in MAP. After increasing VCA activity, through elevating MAP by methoxamine, VIP induced an attenuated tachycardia and only a slight fall in MAP. The intracoronary injection of the vehicle of VIP did not influence HR and MAP. In the protocol IV experiments, HR reached a maximum of $175 \pm 14$ beats/min after

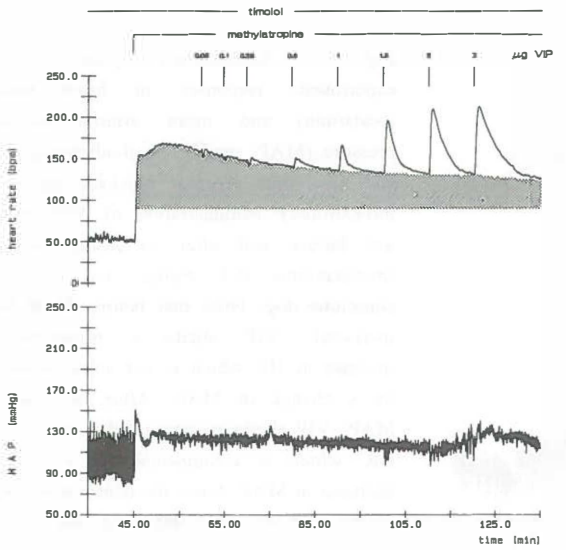



Fig. 5 Representative protocol III experiment: responses in heart rate (beats/min) and mean arterial blood pressure (MAP) (mmHg) to $\beta$-adrenoceptor and muscarinic receptor blockade and to intracoronary injection of increasing doses of VIP (0.05, 0.1, 0.25, 0.5, 1, 1.5, 2 and 3 $\mu$g) in a conscious dog. The VCA activity is represented by the shaded area. The denervated HR (89 beats/min) was determined in a separate experiment following $\beta$-adrenergic and muscarinic receptor blockade after vagotomy on the previous day. Note that VIP elicits a dose-related increase in HR, without producing major changes in MAP.

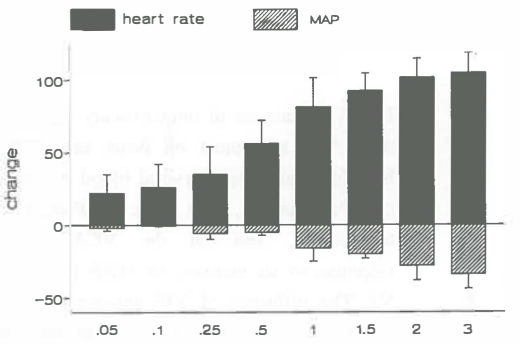



Fig. 6 Responses in heart rate (beats/min) and mean arterial blood pressure (MAP; mmHg) to increasing doses of VIP injected intracoronarily in $\beta$-adrenergic and muscarinic receptor blocked dogs (protocol III; $n=4$).

muscarinic receptors had been blocked. and declined to 143±8 beats/min prior to the first administration of VIP. Following this VIP injection HR increased by 64±13 beats/min (Fig. 2*B*). After stimulation of the $\alpha_1$-adrenoceptors, MAP increased by 101±7 mmHg and HR increased by 66±12 beats/min to a maximum of 204±15 beats/min. After the second VIP injection, HR increased significantly less (by 27±9 to 208±12 beats/min). Following the first VIP injection, MAP hardly changed (-8±2 mmHg), whereas after the second VIP injection a moderate decrease in MAP was observed (-28±4 mmHg). The VIP tachycardia was never followed by a decrease in HR before reaching the previous level, as was observed in some experiments in which VIP was administered intravenously (Fig. 3).

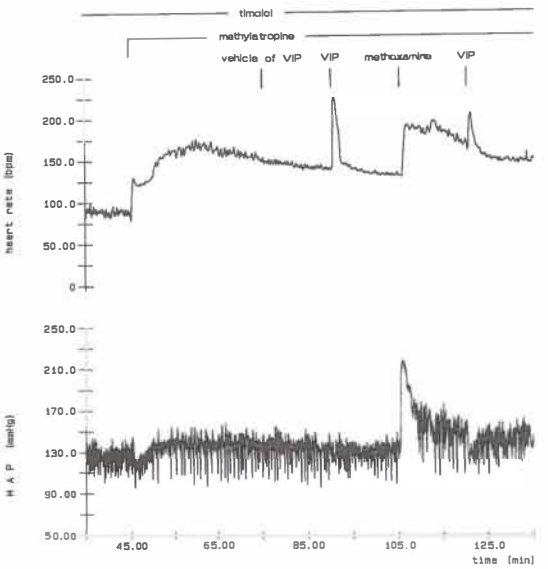



Fig. 7 Representative protocol IV experiment: responses in heart rate (beats/min) and mean arterial blood pressure (MAP; mmHg) to $\beta$-adrenoceptor and muscarinic receptor blockade and to intracoronary administration of VIP (0.5 $\mu$g) before and after increasing MAP (methoxamine 0.1 mg/kg, i.v.) in a conscious dog. Note that before MAP is increased, VIP elicits a pronounced increase in HR which is not accompanied by a change in MAP. After increasing MAP, VIP elicits an attenuated increase in HR, which is accompanied by a slight decrease in MAP. Since the denervated HR of this dog was not determined, the VCA activity could not be quantified and is not represented in the figure.

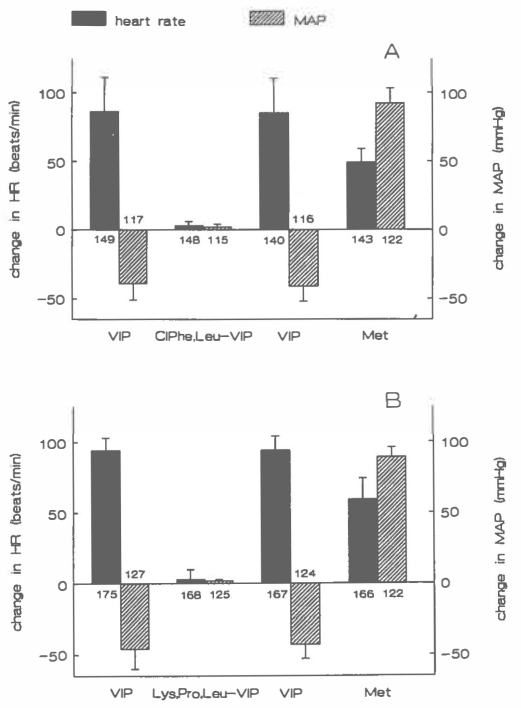



Fig. 8 Influences of intracoronary injection of a VIP antagonist on heart rate (HR; beats/min) and mean arterial blood pressure (MAP; mmHg), on the VIP-induced tachycardia, and on the VCA reflex response to an increase in MAP (protocol V). The influence of VIP antagonist [D-p-Cl-Phe$^6$, Leu$^{17}$]-VIP is displayed in panel A ($n$=5) and that of VIP-antagonist [Lys$^1$, Pro$^{2,5}$, Leu$^{17}$]-VIP in panel B ($n$=4). HR and MAP values prior to the injection of the drugs are reported below or above the bars that represent the response to the intervention.

The administration of the VIP antagonists, [D-p-Cl-Phe$^6$, Leu$^{17}$]-VIP (Fig. 8*A*) and [Lys$^1$, Pro$^{2,5}$, Leu$^{17}$]-VIP (Fig. 8*B*) did not result in changes in HR and MAP. After injection of [D-p-Cl-Phe$^6$, Leu$^{17}$]-VIP or [Lys$^1$, Pro$^{2,5}$, Leu$^{17}$]-VIP, the VCA reflex response to a methoxamine-induced rise in MAP was still pronounced, 49±10 and 59±15 beats/min, respectively (Fig. 8*A* and 8*B*), which was comparable with the VCA reflex responses in protocols I and IV. The VIP tachycardia induced in the presence of [D-p-Cl-Phe$^6$, Leu$^{17}$]-VIP or [Lys$^1$, Pro$^{2,5}$, Leu$^{17}$]-VIP (85±26 and 94±10 beats/min, respectively) did not differ from the VIP tachycardia induced preceding the injection of the VIP antagonists (86±26 and 94±9 beats/min).

**Discussion**

Besides the well known sympathetic cardiac accelerator and the vagal cardiac decelerator system, the existence of a vagal cardiac accelerator system has been described. The activity of the VCA system becomes manifest after blocking the effects of the two classical systems by means of a β-adrenoceptor and a muscarinic receptor antagonist. The terminal transmitter mediating VCA activity has not been established yet. For several reasons VIP has been proposed to produce VCA activity. Several studies in which VIP was brought in relation with VCA system, however, only centered on the chronotropic property of VIP (29, 31), while in but a few studies the relationship between VCA system and VIP was examined (18, 40). However, in these latter studies the relation was only examined by comparing the VIP tachycardia with the vagal cardiac acceleration induced by electric vagal nerve stimulation. The chronotropic property of VIP has never been studied in relation with the *inherent* VCA activity as observed in conscious dogs with blocked β-adrenergic and muscarinic receptors.

In the present study we demonstrated a direct positive chronotropic effect of VIP on the heart in intact and in vagotomized conscious dogs. Superimposed on the VCA activity in the intact dogs, intravenous injection of VIP further increased HR and decreased MAP. The VIP tachycardia is caused by an effect on the sinoatrial node and certainly not reflexly induced by the fall in MAP, because when VIP was injected into the right coronary artery instead of intravenously, a pronounced increase in HR was observed, which was not accompanied a major decrease in MAP. Moreover, after β-adrenoceptor and muscarinic receptor blockade, a decrease in MAP would have reflexly led to a decrease instead of an increase in HR (28, 34). In the present study, this apparently paradoxical reflex response of HR is clearly illustrated by the pronounced increase in HR that follows a methoxamine-induced rise in MAP (Fig. 1, 3 and 7). In

vagotomized animals with blocked β-adrenoceptors and muscarinic receptors, HR remained unchanged after elevating MAP by means of methoxamine, while intravenous injection of VIP resulted in a marked increase in HR, thereby confirming the positive chronotropic effect of VIP.

The coronary cannulation technique, which was developed to allow injection of low doses of drugs directly into the sinus node artery of conscious dogs, thereby precluding reflexly mediated circulatory effects, provided further support for the hypothesis that VIP is a transmitter in the VCA system. The VIP tachycardia was significantly attenuated when the VCA activity had been reflexly increased through increasing MAP. Furthermore, the magnitude of the VIP tachycardia was inversely related to the magnitude of the VCA activity prior to VIP. These findings are most easily explained by assuming that VCA activity is mediated by endogenous VIP and that, when VCA activity is high, fewer receptors are left to interact with exogenous VIP. The findings of Karasawa and coworkers (23), who showed that intracardiac parasympathetic nerve stimulation attenuated the positive chronotropic influence of exogenous VIP on isolated dog heart preparations, are in line with this interpretation. The fact that VIP produces less tachycardia when the baseline HR, *i.e.* VCA activity, is elevated adduces evidence but does not provide direct proof that VIP produces VCA activity, because the tachycardia induced by another agonist would probably also be attenuated by the elevated VCA. However, when the VIP tachycardia would not have been attenuated this would have falsified the hypothesis.

Two additional arguments which make VIP a probable mediator in the VCA system have followed from our study. Firstly, by intracoronary injection of only a small dose of VIP HR already increased to a level similar to that observed during maximum inherent VCA activity. This powerful positive chronotropic effect of VIP has also been established by others in cardiac denervated anesthetized dogs (29), in β-adrenergic, muscarinic and nicotinic receptor blocked conscious dogs (31) and in other mammals including man (9, 27). Secondly, when VIP was injected intravenously an increase in HR was observed which was accompanied by a pronounced decrease in MAP. This increase in HR was similar to that found when VIP was injected intracoronarily, whereas an agonist with a positive chronotropic and a vasodilator effect probably would have produced a moderate biphasic increase in HR, *i.e.* the combination of a direct chronotropic effect of the agonist together with a reflex decrease in VCA activity, induced by the fall in arterial blood pressure.

Also other neuropeptides, including peptide-histidine-isoleucine, secretin, glucagon, neurotensin, neuropeptide Y, substance P and calcitonin gene-related peptide,

have been associated with cardiac control. Although Rigel showed peptide-histidine-isoleucine, secretin and glucagon like VIP produce a pronounced positive chronotropic effect, he assumed that VIP is responsible for the VCA activity, which he designated "excess tachycardia" (28), because the other neuropeptides are less potent than VIP (29). However, to rule out the role of other neuropeptides in VCA activity in dogs, the chronotropic effects of neuropeptides should be investigated by using antagonists for these neuropeptides or by using a coronary cannulation technique, which makes it possible to discriminate between direct and indirect chronotropic effects of the drugs in intact conscious dogs.

There are still other reasons to assume that VIP is associated with the VCA system. The high frequency of electric vagal nerve stimulation that results in the maximum release of VIP from isolated canine heart preparations (20) and from the hearts of intact anesthetized dogs (2) is similar to the frequency required to produce VCA activity (18, 20). In keeping with these findings, Anderson and colleagues (2) reported a 25% increase in VIP content in arterial and coronary sinus blood after stimulating vagal nerves during 3 min with 20 Hz.

Another argument for the VCA activity being mediated by VIP is that the cardiac acceleration (7, 12) and the VIP release (13) during electric vagal nerve stimulation are both suppressed by ganglionic nicotinic receptor blockade. In addition, VIP-immunoreactivity has been found in neuronal elements which are involved in cardiac innervation such as the nucleus dorsalis nervi vagi and the nucleus ambiguus (14) and in the atrial and ventricular myocardium and the sinoauricular and atrioventricular nodes (1, 14, 41). Moreover, VIP receptors have been demonstrated on atrial and ventricular cell membrane preparations (9, 19).

In order to confirm the involvement of VIP in the VCA system, Henning (18) and Wallick et al. (40) studied the influence of the VIP antagonist [D-p-Cl-Phe$^6$, Leu$^{17}$]-VIP on the stimulation induced VCA activity in chloralose anesthetized dogs with blocked $\beta$-adrenoceptors and muscarinic receptors. Henning injected [D-p-Cl-Phe$^6$, Leu$^{17}$]-VIP directly into the coronary artery and stimulated the vagal nerve 20 min after the injection and after having removed the cannula from the coronary artery. In the control situation he found that during 20 Hz vagal nerve stimulation HR increased from 102 to 136 beats/min; after injecting 2 and 4 $\mu$g/kg of [D-p-Cl-Phe$^6$, Leu$^{17}$]-VIP HR increased to 111 and 106 beats/min, respectively. Thus, *stimulation-induced VCA activity* amounted to only 34 beats/min in the control situation and was reduced to 9 and 4 beats/min after 2 and 4 $\mu$g/kg of the VIP antagonist had been injected, respectively. In a similar study, Wallick et al. (40) observed that HR increased from 109 to 154 beats/min, *i.e.* a VCA activity of 45

beats/min, during vagal nerve stimulation before injecting 5 μg/kg of [D-p-Cl-Phe$^6$, Leu$^{17}$]-VIP, while 30 min after the injection vagal nerve stimulation had virtually no effect on HR. In addition, Wallick et al. (40) noticed that after injecting [D-p-Cl-Phe$^6$, Leu$^{17}$]-VIP the reflex VCA response to a blood pressure rise, which was induced by injecting 50 μg of α-adrenoceptor agonist phenylephrine, was abolished and that the increase in HR following intracoronary injection of 0.08 μg/kg of VIP was reduced by 50% of the increment induced before the VIP antagonist had been given. From the findings in these two studies it was concluded that VIP or a VIP-like substance is the principal mediator of VCA activity. To confirm this conclusion we studied the effect of a comparable dose of [D-p-Cl-Phe$^6$, Leu$^{17}$]-VIP on the *inherent* VCA activity, on the reflex VCA response to a rise in MAP and on the VIP-induced tachycardia in conscious dogs. However, comparable doses of [D-p-Cl-Phe$^6$, Leu$^{17}$]-VIP nor the injection of VIP antagonist [Lys$^1$, Pro$^{2,5}$, Leu$^{17}$]-VIP were effective in reducing the prevailing inherent VCA activity or to reduce the VCA reflex response to an increase in MAP. Neither did these VIP antagonists reduce the VIP-induced tachycardia. The discrepancy in results cannot be readily explained. However, there are important differences in experimental design. Firstly, the use of chloralose anesthesia in the studies of Henning (18) and Wallick et al. (40) may have been an interesting factor, since chloralose anesthesia has been shown to reduce VCA activity (8). Actually, the induced VCA activity was only moderate in their experiments, while in our study a maximum HR of 182 beats/min signified a considerably larger VCA activity. Secondly, the chronotropic response to vagal nerve stimulation was studied in intact animals but should have been performed in sympathectomized animals, because the accelerator response to vagal nerve stimulation is inhibited by the sympathetic nervous system (22).

The absence of an effect of the VIP antagonists in our study, however, provides no argument against the involvement of VIP in the VCA system. The observation that the VIP antagonists inhibited the VIP-induced responses in canine kidney (16) and rat central nervous system (15), but did not affect the VIP effects in canine trachea (17), feline airways (37) and canine heart (present study), suggests the existence of different subtypes of VIP receptors. Actually, different VIP receptor subtypes acting through distinct pathways have already been found (19, 32).

The physiological stimuli that cause the release of the transmitter responsible for VCA from the vagal nerve endings remains to be established. According to Hökfelt (21) neuropeptides are not released under basal conditions, but become synthesized in increasing amounts and released only upon activation at high frequency or bursting firing. It is conceivable that the VCA system acts as a braking mechanism, preventing cardiac

arrest during strong vagal decelerator activity, for example during sleep and after stopping strenuous exercise. In addition, the reflex activation of the VCA system in response to increases in MAP on the one hand and the positive chronotropic, inotropic (23, 30) and coronary vasodilator (6, 14, 36, 38) properties of VIP on the other, indicate a possible role in maintaining cardiac output and an appropriate coronary blood flow rate during stress situations. In line with this hypothesis is the finding that HR increased in excess of the denervated HR in response to exercise and emotional stress in sympathectomized (5) and in $\beta$-adrenoceptor blocked (3) dogs. When, in the latter experiments, during submaximal exercise atropine was injected, HR only slightly increased by 15 to 192 beats/min, indicative of a low vagal decelerator activity and a pronounced vagal accelerator activity. On account of the considerably lower HR following muscarinic blockade in $\beta$-adrenoceptor blocked dogs with experimental heart failure in comparison to normal dogs (39), the decreased myocardial concentration of VIP that has been found in canine cardiomyopathy (30) and the decreased number of VIP receptors which have been detected in the failing human heart (19), the activity of the VCA system and VIP are both associated with cardiac pathophysiology, which supports the hypothesis that VIP is the terminal transmitter in the VCA system.

In conclusion, by using a specially developed coronary cannula, we established the direct positive chronotropic effect of VIP in $\beta$-adrenergic and muscarinic receptor blocked intact conscious dogs. The inverse relation between the VIP tachycardia and the prevailing VCA activity, the absence of a secondary decrease in HR following the VIP-induced fall in blood pressure, and the cardiac distribution of VIP-immunoreactive neurons support the hypothesis that VIP has a function in HR control, and exerts its influence through the VCA system. Ultimate proof for this hypothesis awaits the development of a specific cardiac VIP receptor antagonist.

## References


1. Anderson FL, Hanson GR, Reid B, Thorpe M, Kralios AC (1993) VIP and NPY in canine hearts. Distribution and effect of total and selective parasympathetic denervation. *Am J Physiol* 265: H91-H95
2. Anderson FL, Kralios AC, Reid B, Hanson GR (1993) Release of vasoactive intestinal peptide and neuropeptide Y in canine heart. *Am J Physiol* 265:H959-H965
3. Billman GE, Hoskins RS (1989) Time-series analysis of heart rate variability during submaximal exercise. Evidence for reduced cardiac vagal tone in animals susceptible to ventricular fibrillation. *Circulation* 80:146-157
4. Boehm R (1875) Ueber paradoxe Vaguswirkungen bei curarisierten Thieren; Beiträge zur Kenntniss der Physiologie der Herznerven und der Pharmakologie des Curare. *Arch Exp Pathol Pharmakol* 4:351-373
5. Brouha L, Cannon WB, Dill DB (1936) The heart rate of the sympathectomized dog in rest and exercise. *J Physiol* 87:345-359
6. Brum JM, Bove AA, Sufan Q, Reilly W, Go VLW (1986) Action and localization of vasoactive intestinal peptide in the coronary circulation: evidence for nonadrenergic, noncholinergic coronary regulation. *J Am Coll Cardiol* 7:406-413
7. Brunsting JR, Schuil HA, Zijlstra WG (1983) Intrinsic heart rate in the dog determined by pharmacologic denervation. *Am J Physiol* 245:H592-H597
8. Chassaing C, Boucher M, Breysse C, Duchêne-Marullaz P (1992) Contribution of vagal blockade to the tachycardia induced by the antimuscarinic agents atropine and pirenzepine. *J Auton Pharmacol* 12:359-368
9. Christophe J, Waelbroeck M, Chatelain P, Robberecht P (1984) Heart receptors for VIP, PHI, secretin are able to activate adenylate cyclase and to mediate inotropic and chronotropic effects. Species variations and physiopathology. *Peptides* 5:341-353
10. Corr L (1992) Neuropeptides and the conduction system of the heart. *Int J Cardiol* 35:1-12
11. Dale HH, Laidlaw PP, Symons PT (1910) A reversed action of the vagus on the mammalian heart. *J Physiol* 41:3-18
12. Donald DE, Samueloff SL, Ferguson D (1967) Mechanisms of tachycardia caused by atropine in conscious dogs. *Am J Physiol* 212:901-910
13. Fahrenkrug J (1993) Transmitter role of vasoactive intestinal peptide. *Pharmacol Toxicol* 72: 354-363
14. Forssmann WG, Triepel J, Daffner C, Heym C, Cuevas P, Noble MIM, Yanaihara N (1988) Vasoactive intestinal peptide in the heart. *Ann NY Acad Sci* 527:405-420
15. Gozes I, McCune SK, Jacobson L, Warren D, Moody TW, Fridkin M, Brenneman DE (1991) An antagonist to vasoactive intestinal peptide affects cellular functions in the central nervous system. *J Pharmacol Exp Ther* 257:959-966
16. Griffiths NM, Rugg EL, Simmons NL (1989) Vasoactive intestinal peptide control of renal adenylate cyclase: in vitro studies of canine renal membranes and cultured canine epithelial (MDCK) cells. *Q J Exp Physiol* 74:339-353
17. Hakoda H, Ito Y (1990) Modulation of cholinergic transmission by the peptide VIP, VIP anti-serum and VIP antagonists in dog and cat trachea. *J Physiol* 428:133-154
18. Henning RJ (1992) Vagal stimulation during muscarinic and $\beta$-adrenergic blockade increases atrial contractility and heart rate. *J Auton Nerv Syst* 40:121-130

19. Hershberger RE, Anderson FL, Bristow MR (1989) Vasoactive intestinal peptide receptor in failing human ventricular myocardium exhibits increased affinity and decreased density. *Circ Res* 65:283-294
20. Hill MRS, Wallick DW, Martin PJ, Levy MN (1993) Frequency dependence of vasoactive intestinal polypeptide release and vagally induced tachycardia in the canine heart. *J Auton Nerv Syst* 43:117-122
21. Hökfelt T (1992) Neuropeptides in perspective: the last ten years. *Neuron* 7:867-879
22. Kabat H (1940) The cardio-accelerator fibers in the vagus nerve of the dog. *Am J Physiol* 128: 246-257
23. Karasawa Y, Furukawa Y, Ren L-M, Takei M, Murakami M, Narita M, Chiba S (1990) Cardiac responses to VIP and VIP-ergic-cholinergic interaction in isolated dog heart preparations. *Eur J Pharmacol* 187:9-17
24. Koyama S, Fujita T, Shibamoto T, Matsuda Y, Uematsu H, Jones RO (1990) Contribution of baroreceptor reflexes to blood pressure and sympathetic responses to cholecystokinin and vasoactive intestinal peptide in anesthetized dogs. *Eur J Pharmacol* 175:245-251
25. Meek WJ, Keenan M, Theisen HJ (1929). The auricular blood supply in the dog. *Am Heart J* 4: 591-598
26. Nijmeijer A, Roossien A, Brunsting JR (1994) Permanent cannulation of the right coronary artery in the dog. *Animal Technology* 45:(in press)
27. Playford R, Benito-Orfila MA, Nihoyannopoulos P, Nandha KN, Cockcroft J, Todd S, Ghatei MA, Domin J, Bloom SR, Calam J (1992) Effects of peptide YY on the human cardiovascular system: reversal of responses to vasoactive intestinal peptide. *Am J Physiol* 263:E740-E747
28. Rigel DF, Lipson D, Katona PG (1984) Excess tachycardia: heart rate after antimuscarinic agents in conscious dogs. *Am J Physiol* 246:H168-H173
29. Rigel DF (1988) Effects of neuropeptides on heart rate in dogs: comparison of VIP, PHI, NPY, CGRP, and NT. *Am J Physiol* 255:H311-H317
30. Rigel DF, Grupp IL, Balasubramaniam A, Grupp G (1989) Contractile effects of cardiac neuropeptides in isolated canine atrial and ventricular muscles. *Am J Physiol* 257:H1082-H1087
31. Rigel DF, Lathrop DA (1990) Vasoactive intestinal polypeptide facilitates atrioventricular nodal conduction and shortens atrial and ventricular refractory periods in conscious and anesthetized dogs. *Circ Res* 67:1323-1333
32. Robberecht P, Cauvin A, Gourlet P, Christophe L (1990) Heterogeneity of VIP receptors. *Arch Int Pharmacodyn* 303:51-66
33. Roossien A, Brunsting JR, Zaagsma J, Zijlstra WG (1990) Vasoactive intestinal polypeptide: a probable mediator of vagal cardioacceleration in dogs. *Eur J Physiol* 420[suppl]:R-88
34. Roossien A, Brunsting JR, Nijmeijer A, Zaagsma J, Zijlstra WG (1991) Response of the vagal cardioacceleratory system to pharmacologically induced changes in arterial blood pressure in conscious dogs. *Eur J Physiol* 419:R9
35. Rubino A (1993) Non-adrenergic non-cholinergic neural control of the atrial myocardium. *Gen Pharmacol* 24:539-545
36. Smitherman TC, Dehmer GJ, Said SI (1988) Vasoactive intestinal peptide as a coronary vasodilator. *Ann NY Acad Sci* 527:421-430
37. Thompson DC, Altiere RJ, Diamond L (1988) The effects of antagonists of vasoactive intestinal peptide on nonadrenergic noncholinergic inhibitory responses in feline airways. *Peptides* 9: 443-447

38. Unverferth DV, O'Dorisio TM, Muir III WW, White J, Miller MM, Hamlin RL, Magorien RD (1985) Effect of vasoactive intestinal polypeptide on the canine cardiovascular system. *J Lab Clin Med* 106:542-550

39. Vatner SF, Higgins CB, Braunwald E (1974) Sympathetic and parasympathetic components of reflex tachycardia induced by hypotension in conscious dogs with and without heart failure. *Cardiovasc Res* 8:153-161

40. Wallick DW, Hill MR, Martin PJ, Levy MN (1991) The effect of (4-Cl-D Phe$^{6}$, Leu$^{17}$) VIP on vagally induced and VIP induced tachycardias. *Circulation* 84-II:265

41. Weihe E, Reinecke M, Forssmann WG (1984) Distribution of vasoactive intestinal polypeptide-like immunoreactivity in the mammalian heart. *Cell Tissue Res* 236:527-540

# 7

# REFLEX ACTIVATION OF THE VAGAL CARDIAC ACCELERATOR SYSTEM IN CONSCIOUS DOGS, LAMBS AND RATS


## Abstract

In the present study the vagal cardiac accelerator (VCA) influence was examined in conscious, chronically instrumented dogs, rats and lambs. After $\beta$-adrenoceptor blockade, all species responded to a rise in mean arterial blood pressure (MAP), induced through the injection of the $\alpha_1$-adrenergic receptor agonist methoxamine, with a decrease in heart rate (HR), signifying an enhanced vagal cardiac decelerator (VCD) activity (protocol I). After combined $\beta$-adrenoceptor and muscarinic receptor blockade, all species responded to a rise in MAP with an increase in HR, signifying an augmented VCA activity (protocol II). When in protocol I in dogs and lambs muscarinic receptors were subsequently blocked, HR reached a higher level in comparison with protocol II, signifying an elevated VCA activity. The elevated VCA activity is probably related to the augmented methoxamine-induced increase in MAP following muscarinic receptor blockade in these species. Because an elevation in MAP still led to an increase in HR in $\beta$-adrenergic, muscarinic and ganglionic nicotinic receptor blocked dogs and rats (protocol III), the transmission of the VCA impulse at the parasympathetic ganglion seems to be mediated, at least in part, by a non-cholinergic vagal transmitter. The results clearly show that the vagal cardiac accelerator system is active in the lamb and the rat, as well as in the dog.


## Introduction

Shortly after the cardiac decelerator function of the vagus nerve was established, an additional accelerator influence was discovered. Animals of various species responded to electric vagal nerve stimulation with an increase in heart rate after they had been treated with atropine (7, 29). Although the vagal cardiac accelerator (VCA) system has been demonstrated several times, its existence was also vigorously denied, because in many other studies no accelerator response could be obtained (12, 28). Moreover, many

investigators were reluctant to admit its existence, because the VCA system did not fit into the classical concept of autonomic nervous control, consisting of sympathetic and parasympathetic divisions with functionally opposite effects. The issue of the VCA system then became for a considerable period abandoned, since apparently no physiological significance could be defined, because virtually all attempts to reflexly activate the VCA system failed.

Notwithstanding the fact that Jourdan and Nowak (14) presented rather conclusive evidence for the existence of a VCA system in the dog, and others demonstrated VCA activity following emotional excitement or intense exercise in muscarinic receptor blocked, sympathectomized cats (13) and dogs (3, 17), the VCA system did not receive renewed attention until spontaneous VCA activity was demonstrated following muscarinic receptor blockade in $\beta$-adrenoceptor blocked dogs (4, 8), and more became known about the underlying mechanism and the possible function in cardiovascular control. VCA activity is probably mediated by a neuropeptide released from the terminal endings of cardiac vagus nerves (26). On account of the increase in VCA activity following a rise in blood pressure, the VCA system seems to contribute to the regulation of blood pressure (25, 27). Although spontaneous VCA activity and the relationship between blood pressure and VCA activity has only been examined in dogs, the VCA system may be involved in the baroreflex mechanism in other species as well. To find evidence for this hypothesis, we studied the changes in VCA activity in response to blood pressure elevations in rats and lambs and compared them with those obtained in dogs.

## Material and Methods

Experiments were conducted on 5 mongrel dogs of both sexes (25-36 kg), 2 male lambs (3 month old, weighing 18 kg) and 9 male Wistar rats (12 month old, weighing 420-590 g). The dogs and lambs were kept social; the rats were housed individually in plexiglass cages (25x25x30 cm) on woodshavings. All animals had free access to standard laboratory food and water. The rats were kept on a 12:12 h light-dark regime (20.00-08.00 h light); room temperature was 21°C.

*Surgery in dog and lamb*

In all animals indwelling catheters (Tygon microbore S-54-HL, ID 0.5 mm, OD 1.5 mm) were inserted into the right omocervical vein and artery, and the tips were advanced to the superior caval vein and descending aorta, respectively. Subsequently, the catheters were tunnelled subcutaneously and secured to the skin in the midscapular region.

In the dog, surgery was carried out under neuroleptanesthesia with fentanyl (0.1 mg/kg, i.m.) and fluanisone (5 mg/kg, i.m.) (Hypnorm, Janssen). In the lamb the procedure was performed under ketamine anesthesia (4 mg/kg, i.v.) (Ketalar, Parke-Davis) in combination with diazepam (4 mg/kg, i.v.) (Diazemuls, Dumex). Catheters were kept patent by daily flushing with heparinized saline. The animals wore jackets to protect the catheters from being chewed on. The experiments started after the animals had recovered for at least 14 days; in the meantime the animals were trained to stand quietly in a modified Pavlovian sling.

*Surgery in rat*

To allow frequent infusions in unrestrained and undisturbed freely moving rats, the animals were catheterized according to the method described by Steffens (32). Briefly, a silicon catheter (ID 0.5 mm, OD 1.0 mm) was inserted into the right external jugular vein and advanced to the entrance of the right atrium. For recording of blood pressure and HR the abdominal aorta was catheterized according to the technique of Weeks and Jones (35). A silicon catheter (ID 0.5 mm, OD 1.0 mm) with a "J"-shaped teflon tip (ID 0.38 mm, OD 0.84 mm) was inserted into the abdominal aorta. The end of the catheters were externalized at the top of the skull. Surgery was performed under a mixture of $O_2$ and $N_2O$ (2:1), supplemented with halothane (2%) (Hoechst). The catheters were kept patent by flushing with heparinized saline. The experiments started after seven days of recovery. During the experiment the arterial catheter was connected to a manometer by means of a polyethylene tube (length 400 mm, ID 0.75 mm, OD 1.45 mm). A similar tube was connected to the venous catheter. During the experiment, the animal was kept in its home cage with free access to water but without access to food.

*Experimental protocol*

Each dog and rat was submitted to three experiments, consecutively according to protocols I, II and III. Each lamb was submitted to a protocol I and a protocol II experiment. Each protocol started by blocking the sympathetic accelerator effect by means of the $\beta$-adrenoceptor antagonist timolol and was followed, 30 min later, by muscarinic receptor blockade, thereby blocking the vagal cardiac decelerator (VCD) action. In protocol I, the HR response to an increase in mean arterial blood pressure (MAP) induced prior to muscarinic receptor blockade was measured. In protocol II, the HR response to an increase in MAP induced after muscarinic receptor blockade was measured. The increase in MAP was induced by administration of the $\alpha_1$-adrenoceptor agonist methoxamine. In protocol III, the HR response to ganglionic nicotinic receptor blockade

after combined β-adrenoceptor and muscarinic receptor blockade was examined. To establish whether the ensuing decrease in VCA activity was due to the nicotinic receptor blockade *per se*, as has been assumed (4, 8), or to the concomitant decrease in MAP, the HR response to a subsequent methoxamine-induced increase in MAP was recorded. To ascertain that the chronotropic response to methoxamine was reflexly induced, an additional experiment was performed, in which methoxamine was injected into two β-adrenergic and muscarinic receptor blocked conscious dogs, after bilateral cervical vagotomy had been performed on the day before the experiment.

*Circulatory measurements*

Systemic arterial blood pressure was measured through the implanted catheter in the aorta with a strain-gauge manometer (dog, lamb: Uniflow, Baxter) (rat: Honeywell 130 PC). In rats, HR was calculated from interbeat intervals, which were obtained from the pressure recording. In dog and lamb, a lead II electrocardiogram (ECG) was recorded using Ag/AgCl electrodes. HR was determined from the ECG with a cardiotachometer (ECG module, Philips V 692). All signals were amplified, analog-to-digital converted and fed into a computer for data processing and display.

*Drugs*

The β-adrenergic receptors were blocked with timolol (rat: 0.7 mg; dog, lamb: 0.2 mg/kg and 0.2 mg/kg/h) (Sigma). Methylatropine (rat: 0.2 mg; dog, lamb: 0.5 mg/kg and 0.5 mg/kg/h) (Sigma) was used as a muscarinic receptor blocking agent. Ganglionic nicotinic receptor blockade was achieved by hexamethonium (rat, dog: 20 mg/kg) (Fluka). The $\alpha_1$-adrenergic receptor agonist methoxamine (dog: 0.1 mg/kg; lamb: 0.3 mg/kg; rat: 0.1 mg) (Wellcome) was administered to increase MAP. All drugs were dissolved in an NaCl solution (154 mmol/l) and administered intravenously.

*Data analysis*

VCA activity is defined as HR in excess of the denervated HR in the combined β-adrenoceptor and muscarinic receptor blocked animal. Since the denervated HR was not determined in most of the animals, the lowest HR after combined receptor blockade was used as a reference to calculate VCA activity. In dog and rat, the lowest HR was typically observed after injection of hexamethonium. In case vagotomy was performed, in addition to β-adrenoceptor and muscarinic receptor blockade, the ensuing HR, being the denervated HR, was used to determine VCA activity. The results were expressed as means ± S.E.M. Results were analyzed using Student's paired *t*-test. A $P$ value $\leq 0.05$

was considered significant.

## Results

*Dog* The maximum changes in HR and MAP following methoxamine in protocol I and II are shown in Fig. 1. When in protocol I methoxamine was injected in $\beta$-adrenoceptor blocked dogs, MAP increased by 86±6 to 192±10 mmHg and HR *decreased* by 53±4 to 29±7 beats/min. When in protocol II methoxamine was injected in $\beta$-adrenoceptor and muscarinic receptor blocked dogs, MAP increased by 97±2 to 225±8 mmHg and HR *increased* by 33±12 to 215±7 beats/min. The maximum HR after muscarinic receptor blockade is shown in Fig. 2*A*. When methylatropine was administered after methoxamine was applied in protocol I, HR increased from 61±5 to 218±10 beats/min, whereas when methylatropine preceded application of methoxamine in protocol II, HR increased from 70±6 to 201±16 beats/min, which was lower than obtained in protocol I.

Following muscarinic receptor blockade in protocol I, MAP increased by 58±4 to 197±9 mmHg, which is a significantly larger increase than obtained in protocol II (by 34±5 to 144±9 mmHg), despite the fact that MAP was already elevated prior to the muscarinic receptor blockade due to the $\alpha_1$-adrenoceptor stimulation (Fig. 2*B*). When in protocol III ganglionic nicotinic receptors were blocked in $\beta$-adrenergic and muscarinic receptor blocked dogs, MAP decreased by 54±8 to 59±6 mmHg and HR *decreased* by 18±2 to 128±7 beats/min (Fig. 3). Following subsequent methoxamine injection, MAP increased by 119±11 to 178±13 mmHg and HR *increased* by 17±3 to 145±9 beats/min (Fig. 3).

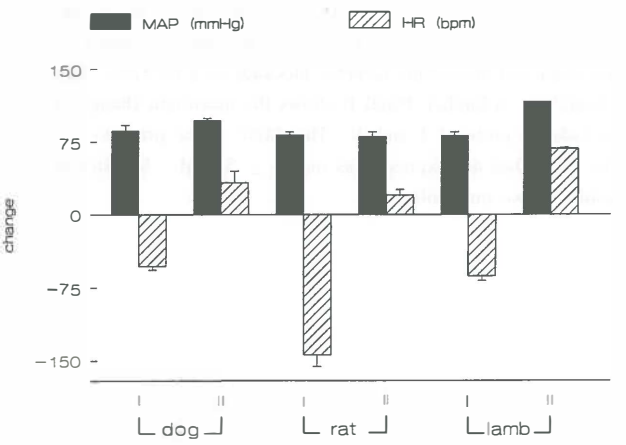



Fig. 1 Maximum changes in heart rate (HR; beats/min) and mean arterial blood pressure (MAP; mmHg) following the administration of methoxamine before (protocol I) and after (protocol II) muscarinic receptors were blocked in $\beta$-adrenoceptor blocked dogs ($n$=5), rats ($n$=9) and lambs ($n$=2). The data are expressed as means ± S.E.M.

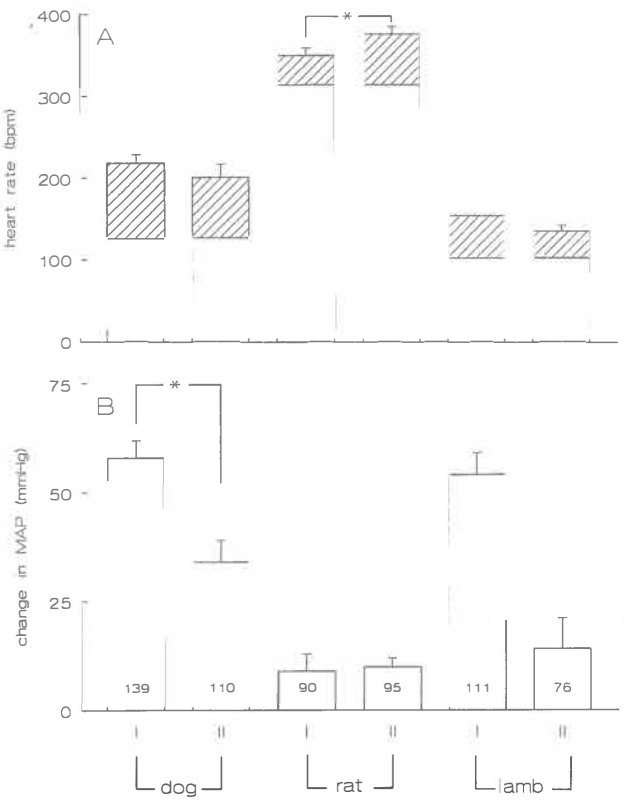



Fig. 2 Maximum heart rate (beats/min) and change in mean arterial blood pressure (MAP; mmHg) following muscarinic receptor blockade in $\beta$-adrenoceptor blocked dogs ($n$=5), rats ($n$=9) and lambs ($n$=2). Panel A shows the maximum HR following combined $\beta$-adrenoceptor and muscarinic receptor blockade in protocol I and II. In protocol I, the animals were treated with methoxamine before muscarinic receptor blockade. Vagal cardiac accelerator activity, represented by the hatched areas, was determined by using the minimum HR after combined $\beta$-adrenoceptor and muscarinic receptor blockade as a reference (128 beats/min in dogs, 315 beats/min in rats, 102 beats/min in lambs). Panel B shows the maximum change in MAP in response to muscarinic receptor blockade in protocol I and II. The MAP value prior to the muscarinic receptor blockade is given within the bars. Data are expressed as means $\pm$ S.E.M. An asterisk indicates a significant difference between the results of two protocols.

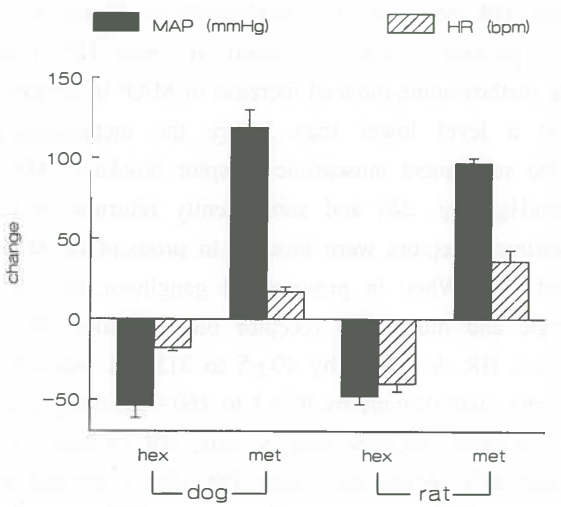



Fig. 3 Maximum changes in heart rate (HR; beats/min) and mean arterial blood pressure (MAP; mmHg) following hexamethonium (hex) and the subsequent administration of methoxamine (met) in β-adrenoceptor and muscarinic receptor blocked dogs ($n$=5) and rats ($n$=9) (protocol III). Values are mean ± S.E.M.

When the minimum HR after combined β-adrenoceptor and muscarinic receptor blockade is taken as a reference, the VCA activity following muscarinic receptor blockade amounted to 218-128=90 and 201-128=73 beats/min in protocol I and II, respectively (Fig. 2*A*; hatched areas). In the additional experiments in the two vagotomized dogs with blocked β-adrenergic and muscarinic receptors, no changes in HR were observed after MAP was increased through the administration of methoxamine. This confirms the reflex and vagal origin of the tachycardia following the injection of methoxamine in intact dogs.

In the vagotomized dogs, with blocked β-adrenergic and muscarinic receptors, HR stabilized at 118 and 84 beats/min, which is considered to be the denervated HR. Prior to vagotomy, in these animals, the lowest HR after combined β-adrenoceptor and muscarinic receptor blockade was 125 and 149 beats/min, respectively. Consequently, the VCA activity actually was 125-118=7 and 149-84=65 beats/min larger than estimated, by taking the minimum HR as the reference level.

*Rat* The maximum changes in HR and MAP to methoxamine in protocol I and II are shown in Fig. 1. When MAP was increased by 82±3 to 179±3 mmHg before muscarinic receptors were blocked (protocol I), HR decreased by 144±12 to 176±10 beats/min. When methoxamine was given after muscarinic receptors were blocked (protocol II) MAP

increased by 80±5 to 183±2 mmHg and HR *increased* by 20±6 to 385±8 beats/min. Following muscarinic receptor blockade, HR increased to a significantly lower maximum (from 285±8 to 350±9 beats/min) in protocol I than in protocol II (from 327±6 to 376±9 beats/min) (Fig. 2*A*). After the methoxamine-induced increase in MAP in protocol I, MAP spontaneously decreased to a level lower than before the methoxamine administration (Fig. 4*A*). Following the subsequent muscarinic receptor blockade, MAP showed a brief increase by 9±4 mmHg (Fig. 2*B*) and subsequently returned to its previous level (Fig. 4*A*). After muscarinic receptors were blocked in protocol II, MAP increased 10±2 mmHg (Fig. 2*B* and 4*B*). When in protocol III ganglionic nicotinic receptors were blocked in β-adrenergic and muscarinic receptor blocked dogs, MAP decreased by 48±5 to 47±4 mmHg and HR *decreased* by 40±5 to 315±11 beats/min (Fig. 3). When MAP was increased using methoxamine by 97±3 to 160±2 mmHg in β-adrenergic, muscarinic and ganglionic nicotinic receptor blocked rats, HR *increased* by 36±7 to 352±9 beats/min (Fig. 3 and 4*C*). When the lowest HR after combined β-adrenoceptor and muscarinic receptor blockade, which was typically observed after injection of hexamethonium (protocol III), was used as a reference to quantify VCA activity, the VCA activity amounted to 350-315=35 beats/min in protocol I (Fig. 2*A*; hatched area) and 376-315=61 beats/min in protocol II (Fig. 2*A*; hatched area).

*Lamb* When MAP was increased through the administration of methoxamine by 81±4 to 153±4 mmHg before muscarinic receptors were blocked (protocol I), HR *decreased* by 63±5 to 38±4 beats/min (Fig. 1). When MAP was elevated by 116±0 to 207±5 mmHg after muscarinic receptors were blocked (protocol II), HR *increased* by 68±1 to 201±9 beats/min (Fig. 1). After muscarinic receptor blockade in protocol I, HR increased from 84±2 to a maximum of 154±0 beats/min (Fig. 2*A*), whereas when the muscarinic receptors were blocked in protocol II, HR increased from 81±6 beats/min to a maximum of 135±7 beats/min. Using the lowest HR after combined β-adrenoceptor and muscarinic receptor blockade (102 beats/min) as a reference, the VCA activity in protocol I amounted to 154-102=52 beats/min (Fig. 2*A*; hatched area) which was larger than VCA activity observed in protocol II: 135-102=33 beats/min (Fig. 2*A*; hatched area). Although in protocol I, MAP was already elevated at the time of the muscarinic receptor blockade (111±5 mmHg) in comparison with protocol II (76±4 mmHg), the increase in MAP following muscarinic receptor blockade was augmented: 54±5 versus 14±7 mmHg in protocol I and II, respectively (Fig. 2*B*). The HR and MAP responses to the pharmacological interventions in protocol I and II in one lamb are displayed in Fig. 5.

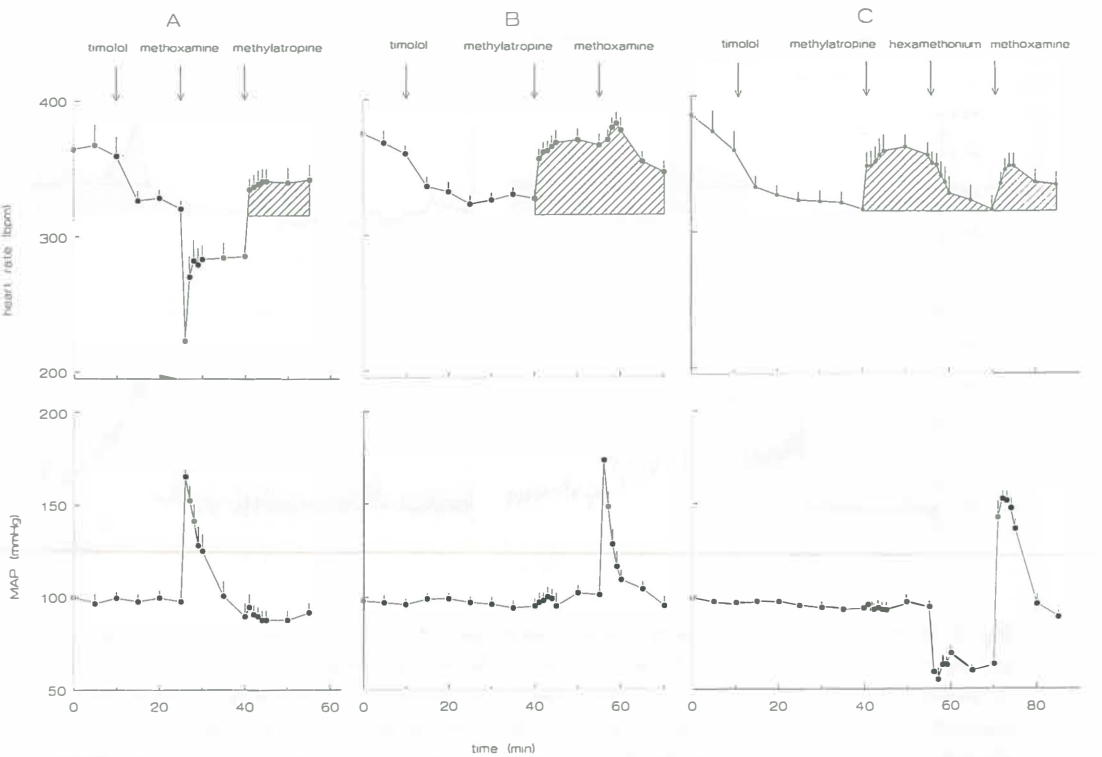



Fig. 4 Heart rate (beats/min) and mean arterial blood pressure (MAP; mmHg) responses to $\beta$-adrenoceptor and muscarinic receptor blockade and reflex changes in HR to alterations in MAP in rats ($n$=9). *A.* shows the HR response to the methoxamine-induced rise in MAP, which was induced before muscarinic receptor blockade (protocol I). *B.* shows the HR response to a rise in MAP, which was induced by methoxamine after muscarinic receptors were blocked (protocol II). *C.* shows the responses in HR and MAP to the successive administration of hexamethonium and methoxamine after muscarinic receptor blockade (protocol III). To illustrate VCA activity, the minimum HR after hexamethonium (315 beats/min) was taken as a reference, because it is closest to the denervated HR. VCA activity is represented by the shaded area. Values are expressed as mean $\pm$ S.E.M.

## Discussion

The existence of the VCA system has been a subject of controversy for a long time, because no VCA activity could be demonstrated in many studies (12), and when it was encountered the underlying mechanism remained obscure (7). After Brouha et al. (3) and Hodes (13) established that the VCA system participates in the chronotropic control of the heart during physical (exercise) and psychological (emotional excitement) stress situations, the physiological role of the VCA system was speculated about for the first

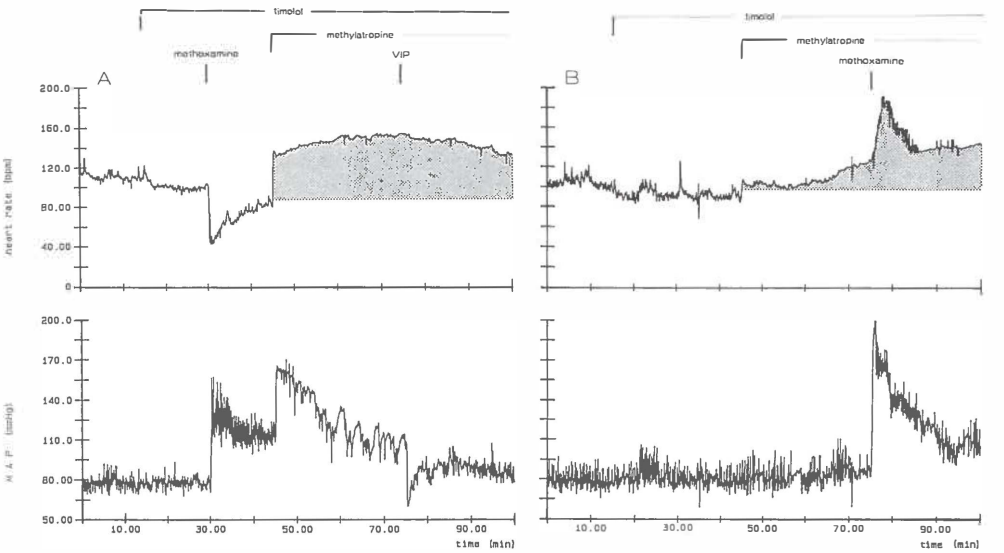



Fig. 5 Heart rate (beats/min) and mean arterial blood pressure (MAP; mmHg) responses to β-adrenoceptor and muscarinic receptor blockade and changes in HR in response to alterations in MAP in one lamb.
*A.* shows the HR response to a rise in MAP, which was induced before muscarinic receptor blockade (protocol I). *B.* shows HR response to a rise in MAP, which was induced after muscarinic receptor blockade (protocol II). In addition, the effect of a bolus (10 μg, i.v.) of vasoactive intestinal polypeptide (VIP) in the β-adrenoceptor and muscarinic receptor blocked lamb is displayed in panel *A.* Note that VIP causes a pronounced decrease in MAP that is not accompanied by an alteration in HR. The minimum HR after combined β-adrenoceptor and muscarinic receptor blockade (98 beats/min) was used as a reference in expressing VCA activity. VCA activity is represented by the shaded area.

time. However, in the following years, attention for the VCA system was lost, because most cardiovascular research was done in anesthetized animals, using anesthetics with strong vagolytic properties. Various kinds of anesthetics have been described to affect cardiac vagal tone and to alter baroreceptor sensitivity (11). In relation to the VCA activity, pentobarbital (4, 8), methoxyflurane (4) and α-chloralose (5) have been reported to suppress spontaneous VCA activity. When these anesthetics are used, VCA activity can only be evoked by electric stimulation of the vagus nerves in atropinized animals (8, 30). However, when neuroleptanesthesia is used in dogs, spontaneous VCA activity can clearly be demonstrated, because this intervention does not suppress vagal tone (4). Nevertheless, neuroleptanesthesia is not the appropriate condition for studying the VCA system, because baroreceptor reflex modulation of the VCA activity that has been encountered in conscious dogs (25, 27) cannot be satisfactorily demonstrated during neuroleptanesthesia

(30). The present study revealed that spontaneous VCA activity, as is typically observed following muscarinic receptor blockade in $\beta$-adrenoceptor blocked conscious dogs, can also be demonstrated in lamb and rat. Because all animals responded to a rise in MAP with an increase in VCA activity, the VCA system may be generally present in mammals and contribute to the baroreflex mechanism. Despite its functionally opposite effects, the vagal cardiac accelerator system is activated under the same circumstance as the vagal cardiac decelerator system. The activation of both systems in response to a blood pressure elevation was demonstrated in $\beta$-adrenoceptor blocked dogs and lambs in protocol I: when MAP was increased before muscarinic receptor blockade, all animals responded with a decrease in HR, signifying an increased VCD activity; after subsequent muscarinic receptor blockade, HR increased to a significantly higher level than was observed when MAP had not been elevated before muscarinic receptor blockade (protocol II), thus demonstrating an enhanced VCA activity. The augmented VCA activity in protocol I is probably related to the elevated blood pressure prior to muscarinic receptor blockade and the enhanced increase in MAP following muscarinic receptor blockade in these cases. In protocol I experiments in rats, HR reached a significantly lower maximum than in protocol II, which fits the fact that prior to muscarinic receptor blockade, MAP was slightly lower in protocol I than in protocol II.

That the VCA system is involved in the regulation of blood pressure has for the first time been demonstrated in conscious dogs by Rigel and co-workers (25), who observed an increase in VCA activity following an elevation in blood pressure and a decrease following a decrease in blood pressure. Later on, Roossien et al. (27) confirmed that the VCA system contributes to the baroreflex mechanism. They showed that $\beta$-adrenoceptor blocked dogs responded to a rise in MAP with an increase in VCD and VCA activity, as shown by the bradycardia observed before and the enhanced tachycardia observed after muscarinic receptors were blocked. The changes were reflexly induced and certainly did not directly result from changes in MAP or from the hemodynamic changes following the administration of methoxamine, because when the vagus nerves were cooled (25) or sectioned (27) no chronotropic changes occurred in response to the administration of the $\alpha_1$-adrenoceptor agonist methoxamine. Moreover, Terzic et al. (33) described that $\alpha_1$-adrenoceptor agonists are devoid of chronotropic effects.

By using the relationship between blood pressure and VCA activity, we were able to clearly demonstrate the existence of the VCA system, in dogs, rats and lambs. Our experiments showed marked species differences in terms of the prevailing activity of the VCA system; dogs exhibited a pronounced spontaneous VCA activity, rats displayed a moderate VCA activity and in lambs only a minor VCA activity was observed (hatched

areas, Fig. 2*A*, protocol II). However, by inducing a rise in arterial blood pressure all animals of these species reflexly responded with a substantial increase in VCA activity.

As to the question whether the VCA system is present in man, its presence in several quite different mammals renders the hypothesis that it is also present in man fairly plausible. Evidence for this hypothesis has been produced by Jose and Collison (15), who reported that *"some healthy subjects responded to β-adrenoceptor and muscarinic receptor blockade with an increase in HR, reaching a level of 160 beats/min, where it remained for 10-15 min, and subsided slowly over the following 20 min. The rhythm appeared from the ECG to be sinus"*. This report is similar to the description given of VCA activity in dogs (4, 8). In another study in man, Jose et al. (16) reported that HR increased during exercise, while β-adrenoceptors and muscarinic receptors were blocked. This increase in HR may correspond with the vagally mediated increase in HR found by others in sympathectomized cats (13) and dogs (3). Hence, we assume that the data obtained in our animal study may also be applicable to man.

Unexpected was the finding in protocol III that a rise in MAP, which was induced after β-adrenergic, muscarinic and ganglionic nicotinic receptors had been blocked, resulted in an increase in HR. Up to now, it has generally been assumed that the ganglionic transmission of the impulse increasing VCA is mediated by nicotinic receptors, because of the disappearance of spontaneous VCA activity after blocking nicotinic receptors in β-adrenoceptor and muscarinic receptor blocked dogs (4), and the lack of a VCA response to vagal nerve stimulation in muscarinic and nicotinic receptor blocked dogs (8). In the present study, ganglionic nicotinic receptor blockade resulted in a decrease in HR and a concomitant decrease in MAP. Because a subsequent methoxamine-induced increase in MAP resulted in an increase in HR, the decrease in HR following nicotinic receptor blockade probably constituted a reduction rather than an abolition of VCA activity, provoked by the concomitant decrease in MAP. That the decrease in HR following nicotinic receptor blockade is, at least in part, a reflex reaction to the alteration in MAP, follows from the observation that methoxamine and the resulting increase in MAP did not produce a change in HR in β-adrenoceptor and muscarinic receptor blocked, vagotomized dogs.

Consequently, combined β-adrenergic, muscarinic and nicotinic receptor blockade is not the functional equivalent of neural ablation. The triple receptor blockade leaves at least one mode of ganglionic transmission intact. This is in agreement with the presence of an accelerator response to vagal nerve stimulation in muscarinic and ganglionic nicotinic receptor blocked dogs as observed by Antonaccio (2). McWilliam and Woolley (21) suggested that in rabbits the non-myelinated preganglionic vagal fibers contain non-

cholinergic transmitters, because hexamethonium did not abolish excitation of the postganglionic vagal fibers following preganglionic vagal nerve stimulation. Furthermore, the existence of "late slow excitatory postsynaptic potentials" has been detected in postganglionic sympathetic nerve fibers, which are probably induced by peptides (18) and it is conceivable that similar potentials are present in the parasympathetic nerve fibers, since preganglionic parasympathetic nerve fibers have been described to contain regulatory peptides (23). Hence, the transmission of the VCA impulse at the level of the ganglia may, at least in part, be mediated by one of these peptides.

The combination of $\beta$-adrenoceptor and muscarinic receptor blockade has often been employed in attempts to determine intrinsic HR in various animals (4, 10, 19) or to establish the effect of drugs on cardiac pacemaker cells, e.g. angiotensin II (19), vasopressin and phenylephrine (6). In clinical studies, intrinsic HR has often been determined to provide insight in the functional state of the heart, in particular that of the sinus node. A few studies in $\beta$-adrenoceptor and muscarinic receptor blocked humans, revealed that intrinsic HR decreased with advancing age (15, 22), decreased in response to exercise training (31) and increased during exercise (16), while in patients with heart disease (9) and sinus node dysfunction (22), intrinsic HR appeared to be decreased. However, when the existence of the VCA system is taken into account, HR after combined $\beta$-adrenoceptor and muscarinic receptor blockade does not constitute the IHR, but signifies the remaining influence of the autonomic nervous system on the sinus node cells, exerted through VCA system. Consequently, the reaction to $\beta$-adrenergic and muscarinic receptor blockade does not give information on the functional state of the heart *per se*. It may, however, have some pathophysiological significance, because different types of cardiovascular disease, including coronary artery disease (1) and myocardial infarction (24), are associated with impaired vagal control. That an alteration in VCA activity may signify cardiovascular disease can also be concluded from the results of Vatner et al. (34) and of Machado and Brody (20), although this has not been recognized by the authors. In the former study in conscious dogs, HR stabilized at a higher level ($187\pm12$ beats/min) after blocking $\beta$-adrenoceptor and muscarinic receptors in the control situation than after experimental heart failure had been induced ($127\pm7$ beats/min). We believe that these dogs displayed a pronounced VCA activity in the control situation and that VCA activity decreased in response to the experimental heart failure. In the latter study, HR stabilized at a higher level after combined receptor blockade in renal hypertensive rats than in normotensive rats. After sino-aortic deafferentation and after electrolytic lesion of the nucleus ambiguus, which caused a reduction in central parasympathetic activity, the HR stabilized at a lower level after combined receptor

blockade (20).

In conclusion, we have adduced evidence for the involvement of a vagal accelerator system in chronotropic control of the heart in dog, rat and lamb. On account of the reflex responses of the VCA system to alterations in MAP, we suggest that the VCA system is generally present and contributes to the baroreflex mechanism. Because VCA activity increased following a rise in MAP in muscarinic and nicotinic receptor blocked animals, we assume that the transmission of the VCA impulse in the parasympathetic ganglia is, at least in part, mediated by vagal non-cholinergic transmission.

## References


1. Airaksinen KEJ, Niemelä MJ, Ikäheimo MJ, Huikuri HV, Linnaluoto MK, Takkunen JT (1991) Effect of coronary arterial occlusion on vagal control of heart rate. *Int J Cardiol* 30:269-274
2. Antonaccio MJ (1974) Paradoxical vagal tachycardia in ganglion-blocked anesthetized dogs. *Eur J Pharmacol* 28:59-65
3. Brouha L, Cannon WB, Dill DB (1936) The heart rate of the sympathectomized dog in rest and exercise. *J Physiol* 87:345-359
4. Brunsting JR, Schuil HA, Zijlstra WG (1983) Intrinsic heart rate in the dog determined by pharmacologic denervation. *Am J Physiol* 245:H592-H597
5. Chassaing C, Boucher M, Breysse C, Duchêne-Marullaz P (1992) Contribution of vagal blockade to the tachycardia induced by the antimuscarinic agents atropine and pirenzepine. *J Auton Pharmacol* 12:359-368
6. Courtice GP, Kwong TE, Lumbers ER, Potter EK (1984) Excitation of the cardiac vagus by vasopressin in mammals. *J Physiol* 354:547-556
7. Dale HH, Laidlaw PP, Symons CT (1910) A reversed action of the vagus on the mammalian heart. *J Physiol* 41:1-18
8. Donald DE, Samueloff SL, Ferguson D (1967) Mechanisms of tachycardia caused by atropine in conscious dogs. *Am J Physiol* 212:901-910
9. Eckberg DL, Drabinsky M, Braunwald E (1971) Defective cardiac parasympathetic control in patients with heart disease. *N Engl J Med* 285:877-883
10. Evans JM, Randall DC, Funk JN, Knapp CF (1990) Influence of cardiac innervation on intrinsic heart rate in dogs. *Am J Physiol* 258:H1132-H1137
11. Halliwill JR, Billman GE (1992) Effect of general anesthesia on cardiac vagal tone. *Am J Physiol* 262:H1719-H1724
12. Hering HE (1924) Über das angebliche Vorkommen von Acceleransfasern im Halsvagus der Saügetiere. *Pflügers Arch* 203: 100-109
13. Hodes R (1939) Exercise in the sympathectomized cat. *Am J Physiol* 126:171-179
14. Jourdan F, Nowak SJG (1936) Étude expérimentale chez le chien des fibres cardio-accélératrices du vague. *Arch Int Pharmacodyn Ther* 53:121-135
15. Jose AD, Collison F (1970) The normal range and determinants of the intrinsic heart rate in man. *Cardiovasc Res* 4:160-167
16. Jose AD, Stitt F, Collison D (1970) The effects of exercise and changes in body temperature on the intrinsic heart rate in man. *Am Heart J* 79:488-498

17. Kabat H (1940) The cardio-accelerator fibers in the vagus nerve of the dog. *Am J Physiol* 128:246-257
18. Katayama Y, Nishi S (1986) Peptidergic Transmission. In: Karczmar AG, Koketsu K, Nishi S (Eds) *Autonomic and Enteric Ganglia; Transmission and its Pharmacology*; Plenum Press, New York and London, p. 181-200
19. Machado BH, Krieger EM, Salgado HC (1987) Changes of the intrinsic heart rate during the onset of renal hypertension. *J Hypertens* 5:755-759
20. Machado BH, Brody MJ (1989) Contribution of neurogenic mechanisms to control of intrinsic heart rate. *Am J Physiol* 256:R231-R235
21. McWilliam PN, Wooley DC (1990) The effect of supranodose vagotomy on the hexamethonium resistent bradycardia in the anaesthetized rabbit. *J Auton Nerv Syst* 29:227-230
22. Marcus B, Gilette PC, Garson Jr A (1990) Intrinsic heart rate in children and young adults: An index of sinus node function isolated from autonomic control. *Am Heart J* 119:911-916
23. Morris JL (1989) The cardiovascular system. In: Holmgren S (Ed) *Comparative physiology of regulatory peptides*; Chapman and Hall, London, p. 273-307
24. Niemelä MJ, Airaksinen KEJ, Ikäheimo MJ, Groundstroem K, Linnaluoto MK, Takkunen JT (1989) Impaired vagal heart rate control after myocardial infarction. *Int J Cardiol* 24:305-309
25. Rigel DF, Lipson D, Katona PG (1984) Excess tachycardia: heart rate after antimuscarinic agents in conscious dogs. *Am J Physiol* 246:H168-H173
26. Rigel DF (1988) Effects of neuropeptides on heart rate in dogs: comparison of VIP, PHI, NPY, CGRP, and NT. *Am J Physiol* 255:H311-H317
27. Roossien A, Brunsting JR, Nijmeijer A, Zijlstra WG (1991) Response of the vagal cardioacceleratory system to pharmacologically induced changes in arterial blood pressure in conscious dogs. *Eur J Physiol* 419:R9
28. Rossbach MJ (1875) Weitere Untersuchungen über die physiologischen Wirkungen des Atropin und Physostigmin. *Arch Gesam Physiol* 10:383-464
29. Rutherford W (1869) Influence of the vagus upon the vascular system. *J Anat Physiol* 3:402-416
30. Schuil HA (1987) *Vagale cardioacceleratie en denervatie van het hondehart*. Thesis, University of Groningen, Van Denderen, Groningen, The Netherlands
31. Smith ML, Hudson DL, Graitzer HM, Raven PB (1989) Exercise training bradycardia: the role of autonomic balance. *Med Sci Sports Exerc* 21:40-44
32. Steffens AB (1969) A method for frequent sampling of blood and continuous infusion of fluids in the rat without disturbing the animal. *Physiol Behav* 4:833-836
33. Terzic A, Pucéat M, Vassort G, Vogel SM (1993) Cardiac $\alpha_1$-adrenoceptors: an overview. *Pharmacol Rev* 45:147-175
34. Vatner SF, Higgins CB, Braunwald E (1974) Sympathetic and parasympathetic components of reflex tachycardia induced by hypotension in conscious dogs with and without heart failure. *Cardiovasc Res* 8:153-161
35. Weeks JR and Jones JA (1969) Routine direct measurement of arterial pressure in unanaesthetized rats. *Proc Soc Exp Biol Med* 104: 646-648

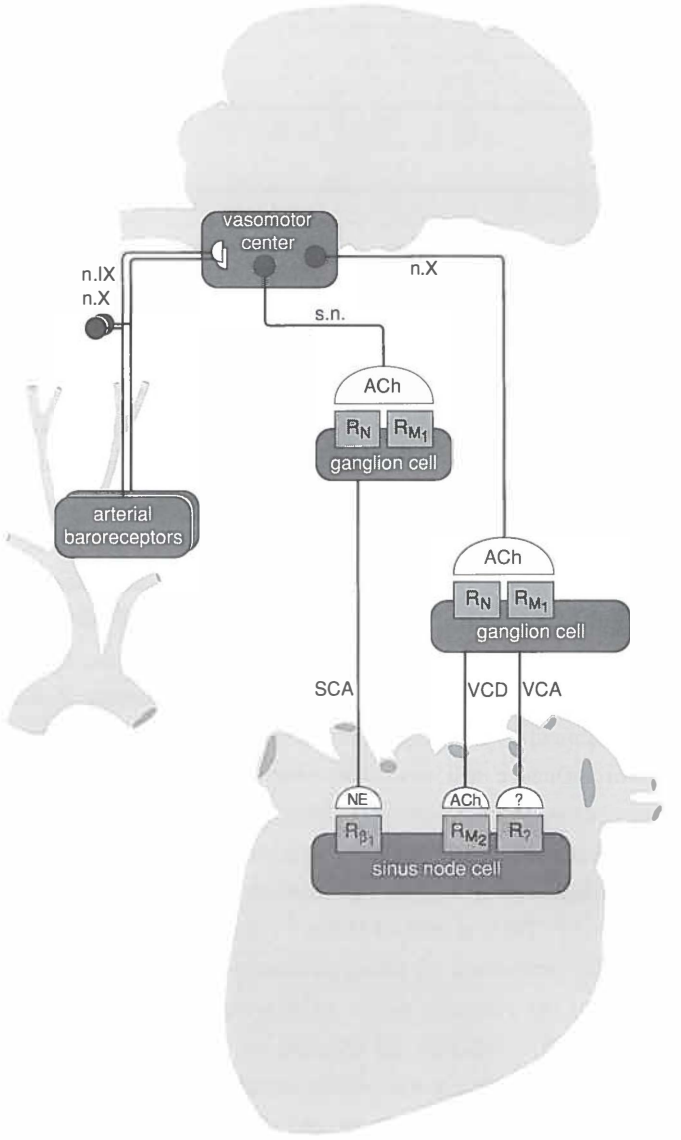
# 8

## GENERAL DISCUSSION

Normal sinus node function results from a balanced interaction between the intrinsic activity of the node and extrinsic factors among which the role of the autonomic nervous system (ANS) is of paramount importance. The ANS controls the chronotropic state of the heart through its sympathetic and parasympathetic divisions. Classically, the sympathetic division exerts an accelerator influence on the sinus node cells by activating $\beta$-adrenergic receptors, and the parasympathetic division exerts a decelerator influence through activating muscarinic receptors. For this reason combined $\beta$-adrenergic and muscarinic receptor blockade has often been employed in attempts to make a distinction between intrinsic and extrinsic influences on the sinus node. However, it has been demonstrated that after combined $\beta$-adrenergic and muscarinic receptor blockade, a vagally mediated accelerator influence still modulates sinus node activity (Chapt. 1: Fig. 1). The existence of a Vagal Cardiac Accelerator (VCA) pathway, however, has not been commonly acknowledged, because most studies concerning heart rate control are performed in anesthetized animals and it has been established that during various kinds of anesthesia, e.g. pentobarbital (12, 24) and methoxyflurane (12), no VCA activity becomes manifest due to the vagolytic properties of these anesthetics. Moreover, many investigators were reluctant to admit the existence of the VCA system, because no physiological significance could be defined - virtually all attempts to reflexly activate the VCA pathway failed - and because the underlying mechanism remained obscure. The studies in this thesis were designed to define the VCA pathway, and to obtain a better understanding of the mechanisms underlying VCA activity. In the following discussion the major findings are reviewed. Furthermore, possible links between VCA activity and heart disease are explored.

### 8.1. The VCA system contributes to the baroreceptor reflex

Arterial baroreceptor reflex-modulation of heart rate is an important component in the short term regulation of arterial blood pressure. In studying the baroreceptor reflex-mediated adjustments in autonomic nervous activity, only the importance of the




Blood pressure increase → activity

| | SCA | VCD | VCA | heart rate | net |
|---|---|---|---|---|---|
| unblocked | ↓ | ↓ | ↑ | | ↓ |
| β block | - | ↓ | ↑ | | ↓ |
| β + M block | - | - | ↑ | | ↑ |

sympathetic cardiac accelerator (SCA) and vagal cardiac decelerator (VCD) pathways are usually taken into account (32, 34, 49). Stimulation of the baroreceptors typically induces a decrease in SCA activity and an increase in VCD activity, both effecting a decrease in heart rate. The possible role of the VCA pathway in the baroreceptor reflex has commonly been overlooked. However, as is described in Chapt. 4, VCA activity has been demonstrated to play a part in the baroreceptor reflex mechanism. It was established that in the presence of the $\beta$-adrenergic receptor antagonist timolol and the muscarinic receptor antagonist methylatropine, heart rate reflexly *increased* in response to an *increase* in arterial blood pressure, and conversely, heart rate reflexly *decreased* in response to a *decrease* in arterial blood pressure. These seemingly paradoxical reflex changes in heart rate in response to alterations in arterial blood pressure have for the first time been associated with a vagal accelerator pathway by Rigel et al. (68), who described that the vagal accelerator influence was enhanced in response to a rise and was attenuated in response to a fall in arterial blood pressure. They speculated that the vagal efferent fibers by means of acetylcholine produce cardioinhibition as well as cardioacceleration.

Fig. 1 Schematic representation of the response of the sympathetic cardiac accelerator (SCA), vagal cardiac decelerator (VCD) and vagal cardiac accelerator (VCA) division of the autonomic nervous system to an increase in arterial blood pressure. The information of the arterial baroreceptors is transmitted through the vagal (n.X) and glossopharyngeal (n.IX) afferent nerves to the vasomotor center in the medulla oblongata. The efferent information of the vasomotor center is transmitted to the sinus node cells via the sympathetic and vagal efferent nerves (s.n. and n.X, respectively). ACh and NE represent the classical neurotransmitters acetylcholine and norepinephrine. $R_N$ and $R_{M1}$ are, in respective, the nicotinic and $M_1$-muscarinic receptors which are localized on the membranes of sympathetic and vagal ganglion cells. $R_{\beta 1}$ and $R_{M2}$ are $\beta_1$-adrenergic and $M_2$-muscarinic receptors which are localized on the membranes of the sinus node cells.
? and $R_?$ represent the unknown terminal transmitter and terminal receptor in the VCA system. Upon an increase in arterial blood pressure, the SCA activity decreases, thereby inducing a decrease in heart rate, unless the $\beta$-adrenergic receptors are blocked ($\beta$-block). Upon an increase in blood pressure, the VCD activity increases. The VCD system induces a decrease in heart rate, unless the muscarinic receptors are blocked (M-block). Upon an increase in blood pressure, the VCA activity increases and induces an increase in heart rate. The influence of the VCA system is normally masked by the influence of the SCA and VCD systems, as indicated by the net decrease in heart rate in the unblocked and $\beta$-adrenoceptor blocked condition, but becomes manifest after combined $\beta$-adrenergic and muscarinic receptor blockade, as indicated by the net increase in heart rate.

We have demonstrated that the VCD and VCA pathways are indeed activated in response to a single stimulus; dogs with blocked β-adrenoceptors responded to a rise in arterial blood pressure with a reflex bradycardia, signifying an increase in VCD activity, while the heart rate increase that followed subsequent muscarinic receptor blockade was *augmented* in comparison with the heart rate increase that followed muscarinic receptor blockade when the arterial blood pressure had not been elevated before, signifying an enhanced VCA activity (Fig. 1). Conversely, β-adrenoceptor-blocked dogs responded to a fall in arterial blood pressure with attenuated VCD and VCA activities, as shown by the tachycardia observed before and the *attenuated* heart rate increase observed after muscarinic receptor blockade. Apparently, the magnitude of the VCA activity that becomes manifest after combined β-adrenergic and muscarinic receptor blockade reflects the extent to which the VCA influence of the ANS is at the moment involved in cardiovascular control.

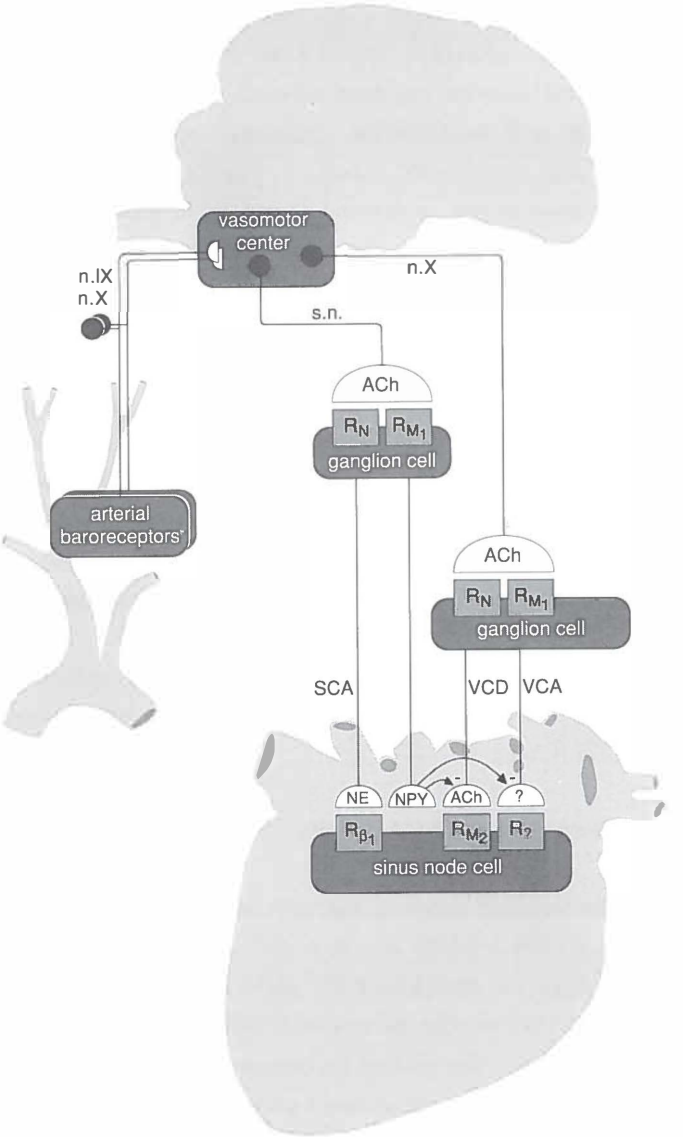
This may also be relevant in connection with the large range of variation in VCA activity that exists between dogs, whereas within each dog there is little variation in VCA activity (Chapt. 4). Hence, each dog seems to have a characteristic tonic vagal efferent activity. In studying VCA activity in dogs, Schuil (77) found that 15% of his animals did not exhibit VCA activity after blocking adrenergic and muscarinic receptors. In some studies from other laboratories, data obtained from dogs that consistently exhibited no VCA activity, were excluded from the analysis, because the transient nature of the VCA activity (68) (see paragraph 8.3) and the accelerator response to vagal nerve stimulation (36) (see paragraph 8.4) could not be satisfactorily examined. However, our results obtained from dogs with a low VCA activity have led to a better insight in the mechanism underlying VCA activity. Dogs that almost lacked VCA activity, hardly responded to a decrease in arterial blood pressure but exhibited a pronounced increase in heart rate following a blood pressure rise. When in these dogs arterial blood pressure had been elevated after β-adrenoceptor blockade and *before* blocking muscarinic receptors, a considerable VCA activity showed up *after* the muscarinic receptor blockade and a prominent decrease in heart rate was observed when subsequently the arterial blood pressure was decreased. Conversely, dogs with a high VCA activity, most prominently reacted to a fall in arterial blood pressure, but showed only a slight increase in heart rate following a blood pressure rise (Chapt. 6: Fig. 3). When in these dogs arterial blood pressure had been lowered *before* muscarinic receptors were blocked, an attenuated VCA activity became manifest *after* muscarinic receptor blockade, and a pronounced increase in heart rate was observed when the arterial blood pressure was elevated subsequently.

Nonetheless, the earlier statement that the maximum heart rate after combined $\beta$-adrenergic and muscarinic receptor blockade reflects the VCA activity prior to muscarinic receptor blockade holds good but to a certain extent, because the change in arterial blood pressure associated with the muscarinic receptor blockade simultaneously modulates the VCA activity. Thus, muscarinic receptor blockade does not simply unmask the prevailing VCA activity, as has been asserted previously (76). That the magnitude of the VCA activity is related to the concomitant change in arterial blood pressure is supported by the finding that in dogs whose blood pressure had been elevated before muscarinic receptor blockade, the enhanced VCA activity following muscarinic receptor blockade was accompanied by an increase in blood pressure (Chapt. 4: Fig. 6), while in dogs whose blood pressure had been lowered before muscarinic receptor blockade, the attenuated VCA activity following muscarinic receptor blockade was accompanied by a decrease in arterial blood pressure (Chapt. 3: Fig. 2*A*). The activation of the baroreceptor reflex following muscarinic receptor blockade is further demonstrated by the concomitant decrease in sympathetic nerve activity as indicated by the decrease in catecholamine concentrations (72). Baroreceptor reflex modulation of VCA activity is probably also responsible for the typical biphasic nature of the increase in heart rate that follows the administration of the muscarinic antagonist. The secondary increase probably signifies the slowly developing increase in VCA activity evoked by the increase in arterial blood pressure.

Although it has been demonstrated that the VCA activity encountered during neuroleptanesthesia and in the conscious state compare reasonably well (11, 12, 77), neuroleptanesthesia is not the appropriate condition for studying the VCA system, because baroreceptor reflex modulation of the VCA activity cannot be satisfactorily demonstrated during neuroleptanesthesia (77) (Chapt. 3).

## 8.2. Transient nature ("Fade") of the VCA activity

In various studies, the transient nature of the VCA activity has been reported (9, 24, 68, 77) (Chapt. 2). VCA activity typically decays to 20% after 70 min and is entirely dissipated by 2 h when the dogs are conscious (68), while during neuroleptanesthesia, VCA declined 80% within 3 h after muscarinic receptors had been blocked (Chapt. 2). Peripheral factors are associated with the gradual decline in VCA activity, because the maximum accelerator response to electric vagal nerve stimulation which occurred after VCA activity had almost completely vanished, was smaller in comparison with the response to a similar stimulation when the VCA activity was at its maximum level


vasomotor center
n.IX
n.X
n.X
s.n.
ACh
$R_N$ $R_{M_1}$
ganglion cell
arterial baroreceptors
ACh
$R_N$ $R_{M_1}$
ganglion cell
SCA
VCD
VCA
NE
NPY
ACh
?
$R_{\beta_1}$
$R_{M_2}$
$R_?$
sinus node cell

(Chapt. 2). The relative role of the interacting peripheral factor that is involved in the expression of the inherent VCA activity and in the accelerator response to vagal nerve stimulation, seems to be related to arterial blood pressure, because dogs that were characterized by a virtual absence of VCA activity and almost lacked an increase in heart rate during electric vagal nerve stimulation (Chapt. 2), showed a profound increase in VCA activity when arterial blood pressure was elevated (Chapt. 4), even when this increase in blood pressure was induced 2 h after the muscarinic receptors had been blocked (Chapt. 6: Fig. 1). For this reason, the decline of VCA activity is not simply due to exhaustion of the releasable pool of the transmitter(s) at the vagal nerve terminals.

Neurotransmitters released by the one division of the ANS have been described to exert an inhibiting influence on the release of the terminal transmitters of the other division, an interaction which underlies the phenomenon of accentuated antagonism (50). Therefore, the heart rate response to vagal nerve activity may depend on the background level of sympathetic nerve activity. This kind of transneuronal interaction has clearly been demonstrated in several studies in which the negative chronotropic response to vagal nerve stimulation was inhibited when the sympathetic nerves were stimulated concurrently (60, 61, 58, 85, 86). Furthermore, Yamasaki and associates (89) demonstrated that the chronotropic response to vagal nerve stimulation faded when the sympathetic nerves were intact, but remained unaltered when sympathetic nervous activity was eliminated. Hence, it is conceivable that the release of transmitter mediating VCA is also transneuronally inhibited by sympathetic activity which may cause the fading of VCA activity. In fact, Kabat (44) reported, long before Levy (50) postulated accentuated antagonism, that during vagal nerve stimulation only slight accelerator responses can be observed when the sympathetic nerves have been left intact, whereas excitation of the vagus nerves was much more effective in producing an accelerator response after sympathectomy.

Fig. 2 The interaction between the sympathetic and vagal nervous divisions of the autonomic nervous system in the heart. Postganglionic sympathetic nerve fibers release neuropeptide Y (NPY) from their nerve terminals. NPY exerts its effect on the sinus node activity, and thereby on heart rate, through a presynaptic inhibitor action on the release of acetylcholine (ACh) from vagal cardiac decelerator (VCD) nerve fibers and on the release of the unknown neurotransmitter (?) in the vagal cardiac accelerator (VCA) nerve fibers. Note that NPY does not directly influence the activity of the sinus node cells. For other abbreviations see Fig. 1.

Although he could not explain the mechanism behind the vago-sympathetic nerve interaction, he was aware of the fact that solving the mystery might help to explain why some investigators, such as Jourdan and Nowak (43) and Brouha et al. (9), had no difficulty in demonstrating an accelerator response to vagal nerve stimulation, while others never obtained an accelerator response.

The influence of the sympathetic nervous division on the vagal nervous system in dogs is probably mediated by a neuropeptide that is co-localized with norepinephrine in the sympathetic nerve terminals. Yang and Levy (91) demonstrated that the inhibitor influence on the vagal effect by the sympathetic nervous division increased when the duration of the antecedent sympathetic nerve stimulation was increased. Neuropeptide Y (NPY) is thought to be the sympathetic substance responsible for the transneuronal inhibitor effect on the vagal action (Fig. 2) for the following reasons. Firstly, exogenous NPY, which is devoid of a direct chronotropic effect (70), mimicked the transneuronal inhibition of vagal nerve activity (33, 58, 61, 86, 89). Secondly, NPY-immunoreactivity has been detected in the sympathetic nerve endings (61) and thirdly, NPY is released during high frequency sympathetic nerve stimulation (3, 86).

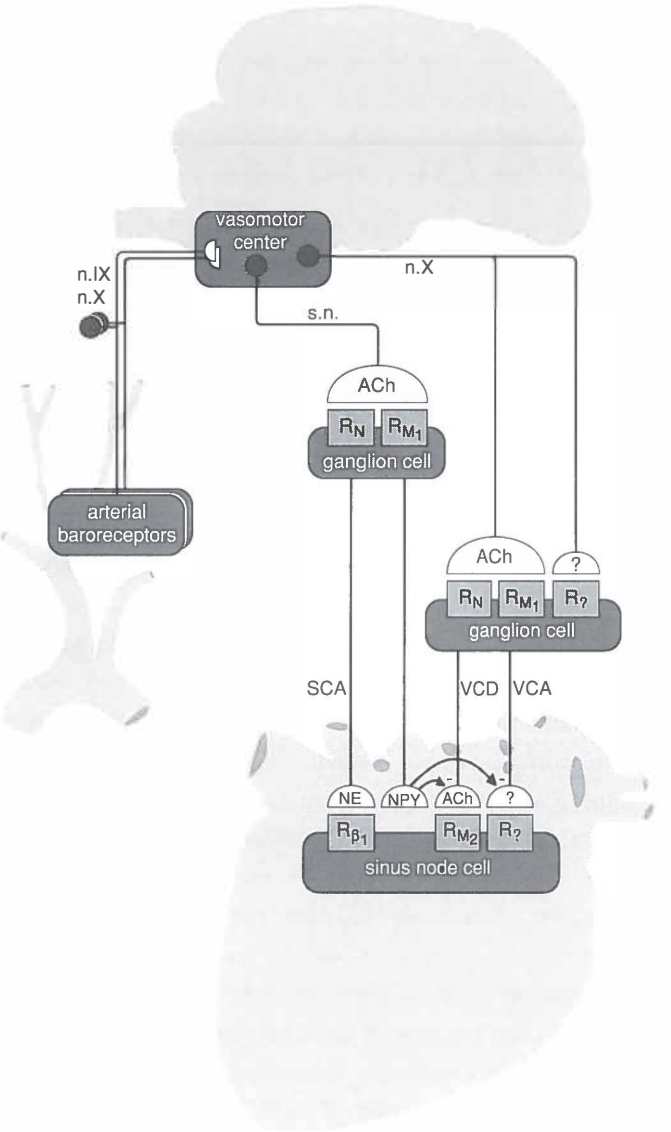
Because the VCA activity also decreased in sympathectomized adrenalectomized dogs (9) and in reserpinized dogs (24), additional factors seem to be involved in the dissipation of VCA activity. Since it has been established that afferent axon collaterals contribute to cardiac chronotropic control, through an intrathoracic cardio-cardiac reflex mechanism (4, 48), and afferent nerve fibers have been identified in intracardiac ganglia, the dissipation of VCA activity may have been brought about through an interaction between afferent nerve fibers and vagal efferent nerve fibers. In this connection it may be relevant that immunoreactivity for several neuropeptides associated with afferent nerve fibers, including substance P (17, 52, 64, 78) and calcitonin gene-related peptide (52, 78), has been detected around ganglion cells in the atrium and that postganglionic vagal nerve activity was modulated when substance P (5, 48) or calcitonin gene-related peptide (48, 88) was injected near the atrial ganglion cells.

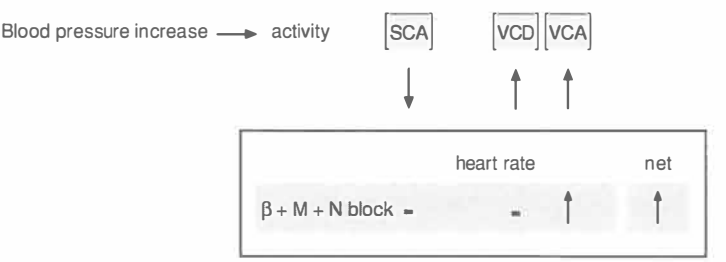
## 8.3. Non-cholinergic ganglionic mediation in the VCA pathway

It is generally assumed that the ganglionic transmission of the VCA impulse is mediated through nicotinic receptors, because in the presence of a ganglionic nicotinic receptor antagonist, dogs with blocked muscarinic receptors failed to increase heart rate in response to vagal nerve stimulation (2, 24) and the VCA activity of $\beta$-adrenoceptor- and muscarinic receptor-blocked dogs disappeared in the presence of nicotinic receptor antago-

nist hexamethonium (12, 76, 77). However, we believe that the transmission of the VCA impulse at the parasympathetic ganglion is mediated, at least in part, by a non-cholinergic transmitter (Fig. 3), because in neuroleptanesthetized dogs with blocked $\beta$-adrenergic and muscarinic receptors, heart rate stabilized at a higher level in the presence of ganglionic nicotinic receptor blocking agent than following vagotomy, signifying that some vagal accelerator influence is still present after nicotinic receptor blockade (Chapt. 2). Furthermore, a rise in arterial blood pressure, which was induced after $\beta$-adrenergic, muscarinic and ganglionic nicotinic receptors had been blocked, resulted in an increase in heart rate (Chapt. 7: Fig. 3), whereas after vagotomy, instead of nicotinic receptor blockade, no chronotropic response was observed following an increase in blood pressure (Chapt. 4: Fig. 7 and 8). Since the heart rate decrease following nicotinic receptor blockade was accompanied by a decrease in blood pressure, it is conceivable that the decrease in heart rate constituted a reflex-mediated reduction in VCA activity instead of an abolition of VCA activity by ganglionic nicotinic receptor blockade. The finding that following additional nicotinic receptor blockade, heart rate decreased to a lower level in the conscious state as compared with the neuroleptanesthetized state may thus be explained by the fact that arterial blood pressure decreased to a larger extent and ultimately stabilized at a lower level when the dogs were conscious (Chapt. 3). Hence, combined muscarinic and nicotinic receptor blockade is not a suitable method for completely blocking ganglionic transmission, and combined $\beta$-adrenergic, muscarinic and nicotinic receptor blockade is not the functional equivalent of neural ablation of the heart. Consequently, double and triple receptor blockades are no satisfactory methods to completely eliminate the influence of the ANS for the determination of the denervated heart rate (12, 27).

Since dogs characterized by a low VCA activity typically displayed insignificant accelerator responses to vagal nerve stimulation, even in the absence of a nicotinic receptor antagonist (Chapt. 2) (36), the lack of an accelerator response after ganglionic nicotinic receptor blockade does not necessarily demonstrate that the nicotinic receptor antagonist blocks the VCA impulse transmission. This is in agreement with our finding that VCA system is still operative despite the presence of a nicotinic receptor antagonist (Chapt. 7). As has already been discussed in paragraph 8.3, peripheral factors, e.g. NPY release from sympathetic nerve terminals, may have inhibited the accelerator response to vagal nerve stimulation. Such a hypothesis, however, implies that the fall in blood pressure due to hexamethonium (Chapt. 3 and 7) elicited an increase in sympathetic activity which has to be transmitted to the heart through the sympathetic ganglia. The application of methylatropine and hexamethonium, however, will also have blocked this transmission. Therefore, our model of the autonomic control of the heart needs the


vasomotor center
n.IX
n.X
n.X
s.n.
ACh
$R_N$ $R_{M_1}$
ganglion cell
arterial baroreceptors
ACh
?
$R_N$ $R_{M_1}$ $R_?$
ganglion cell
SCA
VCD
VCA
NE
NPY
ACh
?
$R_{\beta_1}$
$R_{M_2}$
$R_?$
sinus node cell


Blood pressure increase
activity
SCA
VCD
VCA
heart rate
net
β + M + N block
-
-

addition of non-cholinergic transmission in the sympathetic ganglion (not shown in Fig. 3 and 4). The VCA activity which remained after combined $\beta$-adrenergic, muscarinic and nicotinic receptor blockade may be mediated by non-myelinated vagal nerve fibers, because in several studies in rabbits, it has been found that hexamethonium blocked the impulse transmission of myelinated but not of non-myelinated preganglionic vagal nerve fibers (55), and that the negative chronotropic effect to stimulation of the myelinated vagal nerve fibers was attenuated when the non-myelinated vagal nerve fibers were stimulated concurrently (22).

The existence of a non-cholinergic transmission in the cardiac ganglion is supported by the finding of "late slow excitatory postsynaptic potentials" during electric preganglionic nerve stimulation that were not mediated by acetylcholine, as shown by the fact that the late slow excitatory postsynaptic potentials were not affected by nicotinic and muscarinic receptor antagonists (46). The late slow excitatory postsynaptic potentials have been associated with neuropeptides, since immunoreactivity for neuropeptides, including Vasoactive Intestinal Polypeptide (VIP), substance P, calcitonin gene-related peptide and NPY (5, 17, 29, 48), has been detected in nerve fibers that project to intracardiac ganglia.

Fig. 3 The non-cholinergically mediated impulse transmission in vagal ganglion cells. An increase in arterial blood pressure causes a decrease in sympathetic cardiac accelerator (SCA) activity, and an increase in vagal cardiac decelerator (VCD) and vagal cardiac accelerator (VCA) activity. After combined $\beta$-adrenergic ($\beta$), muscarinic (M) and nicotinic (N) receptor blockade, the changes in SCA and VCD activities have no effect on the heart rate. In contrast, an increased VCA activity results in an increase in heart rate, despite the presence of $\beta$, M and N receptor blocking agents. This can be explained by the presence of an unknown neurotransmitter in preganglionic vagal nerve terminals and that of an unknown receptor on the membranes of vagal ganglion cells. For other abbreviations see Fig. 1 and 2.

## 8.4. Terminal transmitter in the VCA pathway

For the experiments to identify the non-adrenergic, non-cholinergic neurotransmitter underlying VCA activity in dogs, we developed a cannula that can be chronically implanted into the right coronary artery, proximal to the origin of the sinus node artery. By injecting small doses of drugs directly into the right coronary artery of conscious dogs, the chronotropic properties of drugs can be assessed, without baroreceptor reflex-induced alterations in heart rate. The construction of the cannula and the surgical procedure for implantation are described in Chapt. 5.

The functioning of the coronary cannula was tested by means of intracoronary injection of acetylcholine in conscious dogs with blocked $\beta$-adrenoceptors (Chapt. 5). A low dose (0.01 mg) of acetylcholine already caused cardiac arrest for several seconds, without disturbing the dog's behavior. In the presence of $\beta$-adrenergic receptor and muscarinic receptor antagonists, acetylcholine in doses up to 0.5 mg did not influence the prevailing VCA activity, which not only confirms the adequacy of the muscarinic receptor blockade, but also excludes the involvement of cardiac nicotinic receptors in the VCA pathway, as has been postulated by Hoffmann et al. (39) and Haney and Lindgren (34). This finding also constitutes an additional argument for the hypothesis that the decrease in VCA activity following ganglionic nicotinic receptor blockade is due to the fall in arterial blood pressure instead of to the nicotinic receptor blockade *per se* (see paragraph 8.4).

The usefulness of the coronary cannula in establishing the terminal transmitter in the VCA pathway in dogs was shown by the intracoronary administration of histamine (Chapt. 5). Histamine has been proposed as the mediator of VCA activity, because the sinus node is known to be rich in histamine storing mast cells and because histamine exerts a positive chronotropic effect that is not abolished by $\beta$-adrenoceptor blockade (68). However, when we injected small doses of histamine into the right coronary artery of $\beta$-adrenoceptor and muscarinic receptor-blocked dogs, only moderate increases in heart rate were encountered. When the dose of histamine was increased, the heart rate increase remained moderate and was accompanied by a decrease in arterial blood pressure. Furthermore, the heart rate increase to higher doses of histamine became biphasic, which probably resulted from the combination of a direct positive chronotropic effect of histamine and reflex decrease in VCA activity induced by the fall in arterial blood pressure. This makes histamine an improbable candidate for the terminal transmitter in the VCA pathway, which is supported by the finding that the VCA activity which becomes manifest when $\beta$-adrenergic and muscarinic receptors are blocked, can not be blocked by injecting high doses of the histamine $H_1$-receptor antagonist promethazine or

the histamine $H_2$-receptor antagonist cimetidine (69, 77). Moreover, it has been reported that the release of histamine is only slightly increased during vagal nerve stimulation and that this release can be blocked by atropine (7).

VCA activity is assumed to be mediated by neuropeptides because a high frequency of vagal nerve stimulation is required to generate the accelerator response (Chapt. 2) (36, 37), and because the accelerator response is characterized by a long latency, slow onset and long persistence after cessation of the stimulation (Chapt. 2) (24). Evidence has accumulated which indicates that in dogs VIP is the mediator of the VCA effect. From our study it was concluded that VIP exerts a powerful positive chronotropic effect. When VIP was injected intravenously, the VIP-induced tachycardia was accompanied by a marked fall in arterial blood pressure. Several authors, therefore, claimed that the increase in heart rate emanated from the VIP-induced decrease in blood pressure (23, 79). However, when we injected small doses of VIP directly into the coronary artery of $\beta$-adrenergic and muscarinic receptor-blocked conscious dogs, we observed pronounced increases in heart rate which were not accompanied by major decreases in arterial blood pressure (Chapt. 6: Fig. 5). The powerful positive chronotropic effect of VIP in dogs has been confirmed by others in isolated heart preparations (45) and in intact animals (70). In addition, the heart rate increase following high doses of VIP, unlike following a high dose of histamine, remained monophasic, despite the concomitant decrease in arterial blood pressure (Chapt. 6: Fig. 1). That VIP is probably the neurotransmitter in the VCA pathway is supported by the findings that the VIP-induced tachycardia was inversely related to the prevailing VCA activity (Chapt. 6), and similarly, that the VIP-induced tachycardia was attenuated when vagal nerve activity had been increased (Chapt. 6) (45).

VIP has also been brought in relation to the VCA effect because high frequencies of electric stimulation of the vagus nerve are required to result in the release of endogenous VIP (3, 35) and to produce VCA activity (Chapt. 2) (35, 37), while it has been reported that VCA activity (12, 24) (Chapt. 2 and 3) and VIP release (28) are both inhibited in the presence of a nicotinic receptor antagonist. Furthermore, immunoreactivity for VIP has been detected in the vagal centers of the medulla oblongata, *i.e.* dorsal nucleus of the vagus and nucleus ambiguus, in preganglionic vagal nerve fibers (29) and in neurons in the heart, in particular in the sinus node (3, 29, 87). Although it has been suggested that VIP is co-localized with acetylcholine in the vagal nerve terminals (29, 87), others presume that VIP is localized in fibers distinct from those containing acetylcholine (3). Conclusive evidence that VIP is the terminal transmitter in the VCA pathway in dogs has to wait for the availability of a specific cardiac VIP receptor antagonist.

## 8.5. Is the VCA pathway generally present?

Dale et al. (1910) suggested that the VCA pathway exists only in carnivorous animals, because they observed accelerator responses to vagal nerve stimulation in atropinized ferrets, cats and dogs, but not in rabbits (16). In studying VCA activity in conscious animals, only dogs (9, 12, 24, 44, 68) and cats (38, 57) have been used. However, we adduced evidence that the VCA pathway may be generally present in mammals, since we demonstrated VCA activity also in rats and lambs. After blocking $\beta$-adrenergic and muscarinic receptors, the animals of both species responded, in analogy to dogs, to a rise in arterial blood pressure with a seemingly paradoxical increase in heart rate, and conversely (Chapt. 7). Although Machado and co-workers (53) demonstrated that $\beta$-adrenergic and muscarinic receptor-blocked conscious rats respond to an angiotensin II induced blood pressure rise also with an increase in heart rate, these authors interpreted their finding as an increase in intrinsic heart rate, despite the fact that they observed a decrease in heart rate after lesioning the nucleus ambiguus, which indicated the vagal origin of the tachycardia. These authors also demonstrated that during the development of one kidney-one clip hypertension heart rate after combined receptor blockade significantly increased (54).

The VCA pathway has not been investigated in man, but it has been reported that some healthy subjects respond to combined $\beta$-adrenergic and muscarinic receptor blockade with a transient tachycardia that resembles the tachycardia found in dogs (41). Moreover, sudden tachycardia is a known complication following the administration of atropine (26), and it has been demonstrated that vagal nerve stimulation-induced bradycardia is followed by a transient tachycardia (65). Therefore, we assume that the VCA system also exists in man. As a consequence, the decrease in heart rate after combined $\beta$-adrenergic and muscarinic receptor blockade with increasing age as determined in healthy subjects (56) may have indicated a decrease in VCA activity with advancing age instead of a age related decrease of the intrinsic heart rate.

It may be that VCA developed quite early in the development of vertebrates, because various species, including frog (8), ferret (16), rabbit (8, 74), cat (8, 16, 38, 57) and dog (8, 9, 16, 43, 44) (Chapt. 2), respond to vagal nerve stimulation with an increase in heart rate. As discussed in paragraph 8.5, evidence has been adduced that VIP is the mediator of VCA activity in dogs. Although VIP-immunoreactivity has also been detected in cardiac nerve fibers of various other species, including rat, guinea pig, monkey and man (15, 67, 87) and exogenous VIP induces an increase heart rate in these species (23, 30, 40), this does not necessarily imply that in all species VIP is the transmitter mediating

VCA activity, because VIP only slightly increases heart rate in rats (15) and VIP appears to have no chronotropic effect in rabbits (15), cats (80) and lambs (Chapt. 7: Fig. 5). We speculate that neuropeptides which are structurally related to VIP, such as secretin, glucagon, peptide-histidine-isoleucine and helodermin, may be the mediator of VCA activity in other species, because powerful positive chronotropic effects have been demonstrated of secretin in rats (15), of secretin (73) and glucagon in cats (80), and of helodermin in men (71). It may even be assumed that VCA activity is the positive chronotropic effect of several neuropeptides, simultaneously released upon vagal nerve stimulation, because the VIP-related peptides glucagon and peptide-histidine-isoleucine also bring on positive chronotropic effects in dogs (70). However, VIP is assumed to be the main mediator of VCA activity in dogs, because glucagon and peptide-histidine-isoleucine are less potent than VIP (70).

## 8.6. Physiological relevance

In which physiological state and to which extent the VCA pathway is involved in cardiovascular control is still uncertain, because the VCA system acts in concert with the sympathetic accelerator and the vagal decelerator system. As has been demonstrated in the present study, the VCA and VCD systems are activated in response to the same stimulus. Since the VCA system is especially activated in situations of strong vagal nerve activity, it is conceivable that the VCA pathway serves as a gain control, a negative feedback which prevents a situation of cardiac arrest for more than a few seconds. This "escape" function of the VCA system has first been put forward by Dale and co-workers in 1910 (16). The hypothesis is favored by the transient tachycardia that followed the sudden bradycardia when arterial blood pressure was elevated to a level in excess of 200 mmHg in $\beta$-adrenoceptor blocked dogs (Chapt. 4: Fig. 6) and the positive chronotropic effect during electric vagal nerve stimulation in the absence of a muscarinic receptor antagonist (90). That the VCA system is activated in the former situation ensues from the finding that the VCA activity which became manifest *after* additional muscarinic receptor blockade, was enhanced in comparison with the VCA activity obtained in the same dogs when the arterial blood pressure had not been elevated before the muscarinic receptor blockade (Chapt. 4).

As discussed in Chapt. 4, the VCA activity has been suggested to be associated with post-vagal tachycardia, because post-vagal tachycardia, *i.e.* the rebound tachycardia that follows the electric vagal nerve stimulation-induced bradycardia, resembles the late transient tachycardia that follows the early bradycardia in response to a blood pressure

elevation. In contrast to some authors (14, 51), we assume that vagal cardiac acceleration and post-vagal tachycardia share the same underlying mechanism, which is activated in physiological states characterized by a high vagal tone and a slow basal heart rate, e.g. during sleep.

As has been discussed in Chapt. 4 and paragraph 8.2, the VCA system probably serves a function as a hemodynamic control mechanism, because its activity is reflexly activated when blood pressure is elevated and conversely. The VCA pathway may be considered as one of the non-adrenergic, non-cholinergic pathways by which the vagal division of the ANS activates the cardiac system, because in the presence of a muscarinic receptor antagonist, vagal nerve stimulation also produces positive inotropic (35, 62) and positive dromotropic effects (63).

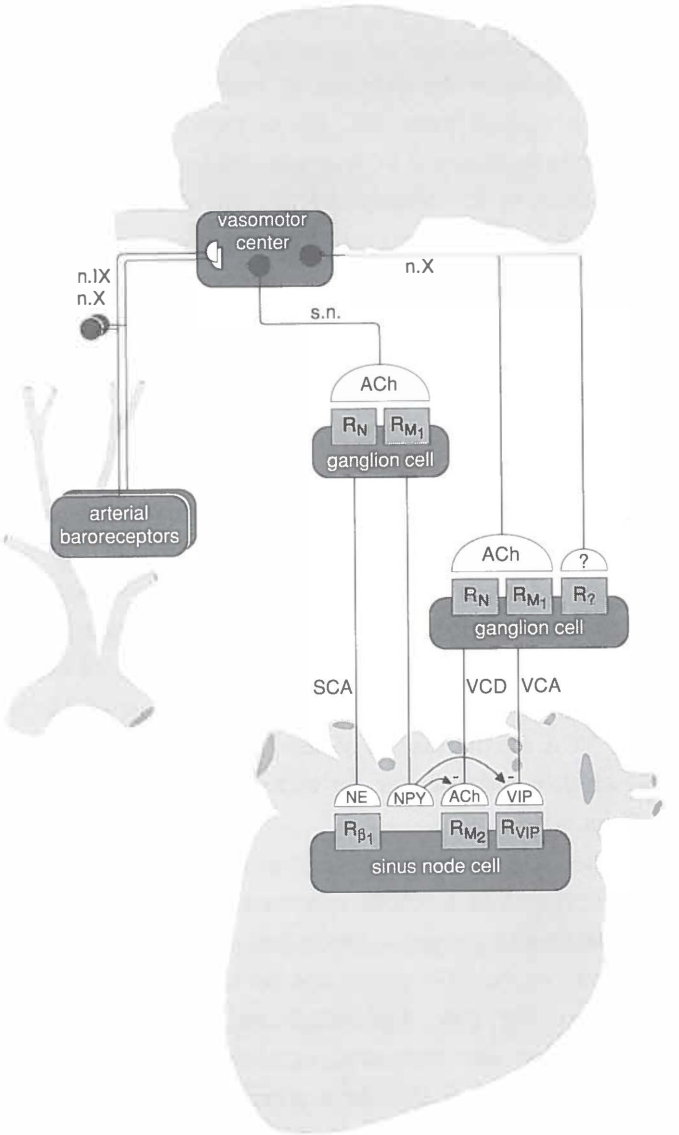
It has also been suggested that the VCA system is activated during exercise, because dogs that lost their sympathetic cardiac accelerator and vagal cardiac decelerator control, showed an increase in heart rate to a level in excess of 200 beats/min during treadmill running (9). This hypothesis is reinforced by the comparable results obtained in sympathectomized, atropinized dogs (75) and cats (38). Hodes (38) demonstrated that the ability to increase heart rate was lost after vagotomy had been performed, thereby confirming the vagal origin of the tachycardia. Billman and Hoskins (6), who were not familiar with the existence of the VCA pathway, more recently observed that dogs with blocked $\beta$-adrenoceptors but with functioning muscarinic receptors, increased their heart rate to a level of about 200 beats/min during treadmill running. The profound vagal accelerator activity during submaximal exercise was apparently accompanied by a low vagal decelerator activity, because heart rate only slightly further increased when the muscarinic receptors were blocked in addition. The VCA system is probably also activated during exercise in man, because men with blocked $\beta$-adrenoceptors and muscarinic receptors also exhibited an increase in heart rate during exercise (42).

Besides in situations of physical stress, the VCA pathway seems also to be activated in situations of psychological stress. Sympathectomized dogs (9, 10) and cats (38) with blocked muscarinic receptors exhibited an increase in heart rate during excitement at the start of treadmill running when the animals were still motionless, while no increase in heart rate was observed when bilateral vagotomy had been performed (10, 38). In a recent study, Randall and co-workers (66) reported that in the presence of a $\beta$-adrenergic and a muscarinic receptor antagonist, all their dogs responded to an aversive conditioned stimulus, *i.e.* a 30-s tone that preceded a shock, with an increase in heart rate. As a possible explanation for the stress-induced tachycardia, they mentioned the release of a non-adrenergic, non-cholinergic substance from vagal nerve fibers.

## 8.7. Clinical implications

Different types of cardiovascular disease, including coronary heart disease (1), ventricular arrhythmia after myocardial infarction (6, 13, 18, 19, 20, 81) as well as hypertension (31, 32), have been associated with a reduced vagal activity and a reduced capability to activate vagal reflexes. On the other hand, increasing vagal nerve activity has been demonstrated to decrease the incidence of ventricular arrhythmia (18, 83), to provide protection against sudden death (20), and to reduce the ischemic injury after coronary artery occlusion (47). That the VCA activity may be of use in establishing the relative role of vagal control in the development of cardiovascular dysfunction may be derived from the results Vatner and colleagues (84) obtained in $\beta$-adrenergic and muscarinic receptor blocked conscious dogs. In their experiments, heart rate stabilized at approximately 180 beats/min in the control situation, while after experimental heart failure had been induced by tricuspid avulsion and pulmonary stenosis, heart rate stabilized at about 125 beats/min, which is the same level that has been described for the denervated heart rate (27). We suggest that a pronounced VCA activity is present in the control situation, while after inducing experimental heart failure VCA activity is suppressed. Furthermore, Stramba-Badiale et al. (82) more recently demonstrated that $\beta$-adrenergic and muscarinic receptor-blocked dogs with a myocardial infarction did not respond to high frequency vagal nerve stimulation with an increase in heart rate. The lack of VCA activity in both studies demonstrates that cardiac dysfunction is associated with an impaired VCA control and thereby emphasizes the significance of the evaluation of VCA activity. In providing information on the parasympathetic nervous influence on the heart the assessment of VCA activity may be of help, because it is a quantitative index of vagal nerve activity, which is characteristic for each individual and reproducible within narrow limits (41) (Chapt. 4).

In clinical studies, the combination of $\beta$-adrenergic and muscarinic receptor blockade has often been employed to obtain information on the functional state of the heart. However, in these studies erroneous conclusions may have been drawn from the results, because the activity of the VCA system was not taken into account. For example, the finding that the heart rate after $\beta$-adrenergic and muscarinic receptor blockade decreases with advancing age was interpreted as a sign of deteriorating sinus node function (21), while it may as well indicate a progressive decrease in VCA activity through a decrease in vagal activity with advancing age. The latter explanation is supported by the decreasing heart rate variability (59) and the attenuation of the heart rate increase following atropine administration (21) with advancing age. In other studies, the


vasomotor center
n.IX
n.X
n.X
s.n.
ACh
$R_N$ $R_{M_1}$
ganglion cell
arterial baroreceptors
ACh
?
$R_N$ $R_{M_1}$ $R_?$
ganglion cell
SCA
VCD
VCA
NE
NPY
ACh
VIP
$R_{\beta_1}$
$R_{M_2}$
$R_{VIP}$
sinus node cell

low heart rate after combined receptor blockade in patients with myocardial disease (25, 56) was attributed to the influence of intrinsic factors on the sinus node activity, while it may have signified an impaired VCA control as well. Hence, in studies in which β-adrenergic and muscarinic receptors have been blocked to investigate cardiac (dys)functioning, a wealth of information may be present on the influence of the VCA system. Furthermore, it may be speculated that the evaluation of VCA activity can be of diagnostic significance in the identification of patients who are at risk to develop, or are already developing, some types of cardiac dysfunction, because very low heart rates became apparent after β-adrenergic and muscarinic receptor blockade in patients with known myocardial disease, many of whom had a normal basal heart rate (41).

## 8.8. Concluding remarks

Although VCA activity was observed for the first time more than a century ago, the existence of the VCA pathway is still not generally acknowledged. However, the findings presented in this thesis clearly indicate that the VCA pathway is involved in the autonomic nervous control of the heart rate. The VCA system presumably plays a part in the regulation of arterial blood pressure, because the VCA system is reflexly activated in response to an elevation in blood pressure. This indicates that the VCA system is associated with the baroreceptor reflex mechanism. Furthermore, it may be speculated that activation of cardiopulmonary and chemoreceptors, which also are involved in circulatory control, may also lead to changes in VCA activity.

Fig. 4 Schematic representation of the autonomic nervous control of the heart rate. This diagram shows, besides the classical sympathetic cardiac accelerator (SCA) and vagal cardiac decelerator (VCD) division, the vagal cardiac accelerator (VCA) division which produces the vagal cardiac acceleration. Following the reflex activation of the vagal cardiac nerves (see paragraph 8.2), the postganglionic VCA fibers release vasoactive intestinal polypeptide (VIP), which activates VIP receptors that are localized on the membranes of the sinus node cells (see paragraph 8.5). The release of VIP from VCA fibers is, like the release of acetylcholine (ACh) from VCD fibers, transneuronally inhibited by neuropeptide Y (NPY) released from postganglionic sympathetic nerve fibers (see paragraph 8.3). Because the vagal cardiac accelerator activity can reflexly be augmented in the presence of a muscarinic receptor ($R_M$) and nicotinic receptor ($R_N$) antagonist, a non-cholinergically mediated impulse transmission in the vagal ganglion is hypothesized (paragraph 8.4). For other abbreviations see Fig. 1, 2 and 3.

Although the VCA system has been studied in but a few animal species, and only extensively in dogs, it is likely that the system is generally present in mammals, because the seemingly paradoxical change in heart rate in response to alterations in arterial blood pressure was also obtained in $\beta$-adrenergic and muscarinic receptor-blocked rats and lambs. Although the VCA system has not been studied in man, the literature contains a lot of data that suggests its existence in man.

On account of the results from our experiments and the findings of others it is very probable that VIP is the mediator of VCA activity in dogs. In other species, however, the VCA activity may be mediated by different substances, because VIP only produces moderate increases in heart rate in the rat and VIP is devoid of chronotropic effects in the cat and the lamb.

In Fig. 4 a model is presented which incorporates the relation between the VCA system and the arterial blood pressure (Chapt. 4, paragraph 8.2), the transneuronal inhibitor influence of the sympathetic nervous division on the vagal outflow (Chapt. 2, paragraph 8.2), the non-cholinergically mediated impulse transmission in the vagal ganglion (Chapt. 7, paragraph 8.3), and VIP as terminal transmitter in the VCA system (Chapt. 6, paragraph 8.4).

To obtain a better insight in the autonomic nervous control of the heart rate in different physiological (exercise, stress, aging) and pathophysiological situations, the determination of the VCA activity may be useful. Dogs with experimental heart failure lacked VCA activity (82, 84) and low vagal nerve activity has been associated with an increased risk of sudden death after myocardial infarction. Furthermore, stimulation of vagal nerve activity has been suggested to represent a novel therapeutic approach to prevent life-threatening arrhythmias in postinfarction patients (13, 19, 20, 81, 83).

## 8.9. References


1. Airaksinen KEJ, Niemelä MJ, Ikäheimo MJ Huikuri HV, Linnaluoto MK, Takkunen JT (1991) Effect of coronary arterial occlusion on vagal control of heart rate. *Int J Cardiol* 30:269-274
2. Alter III WA, Weiss GK, Priola DV (1973) Vagally induced tachycardia in atropinized dogs: effect of $\beta$-adrenergic blockade. *Eur J Pharmacol* 24:329-333
3. Anderson FL, Kralios AC, Reid B, Hanson GR (1993) Release of vasoactive intestinal peptide and neuropeptide Y from canine heart. *Am J Physiol* 265:H959-H965
4. Armour JA (1988) Cardiac effects of electrically induced intrathoracic autonomic reflexes. *Can J Physiol Pharmacol* 66:714-720
5. Armour JA, Bingxiang Yuan, Butler CK (1990) Cardiac responses elicited by peptides administered to canine intrinsic cardiac neurons. *Peptides* 11:753-761
6. Billman GE, Hoskins RS (1989) Time-series analysis of heart rate variability during submaximal

exercise. Evidence for reduced cardiac vagal tone in animals susceptible to ventricular fibrillation. *Circulation* 80:146-157

7. Blandina P, Barattini M, Fantozzi R, Masini E, Mannaioni PF (1983) Histamine release by vagal stimulation. *Agents Actions* 13:179-182
8. Boehm R (1875) Ueber paradoxe Vaguswirkungen bei curarisirten Thieren; Beiträge zur Kenntniss der Physiologie der Herznerven und der Pharmakologie des Curare. *Arch Exp Pathol Pharmakol* 4:351-373
9. Brouha L, Cannon WB, Dill DB (1936) The heart rate of the sympathectomized dog in rest and exercise. *J Physiol* 87:345-359
10. Brouha L, Nowak SJG, Dill DB (1939) The role of the vagus in the cardioaccelerator action of muscular exercise and emotion in sympathectomized dogs. *J Physiol* 95:454-463
11. Brunsting JR, Bennekers JH, Schuil HA, Zijlstra WG (1979) Incomplete cardiac vagal blockade with atropine in the anesthetized dog. *Pflügers Arch* 381:293-295
12. Brunsting JR, Schuil HA, Zijlstra WG (1983) Intrinsic heart rate in the dog determined by pharmacologic denervation. *Am J Physiol* 245:H592-H597
13. Casadei B, Pipilis A, Sessa F, Conway J, Sleight P (1993) Low doses of scopolamine increase cardiac vagal tone in the acute phase of myocardial infarction. *Circulation* 88:353-357
14. Celler BG, Lovell NH (1991) Dynamics of cardiac period responses after prolonged vagal stimulation in the dog. *Ann Biomed Eng* 19:273-289
15. Christophe J, Waelbroeck M, Chatelain P, Robberecht P (1984) Heart receptors for VIP, PHI and secretin are able to activate adenylate cyclase and to mediate inotropic and chronotropic effects. Species variations and physiopathology. *Peptides* 5:341-353
16. Dale HH, Laidlaw PP, Symons CT (1910) A reversed action of the vagus on the mammalian heart. *J Physiol* 41:1-18
17. Dalsgaard CJ, Franco-Cereceda A, Saria A, Lundberg JM, Theodorsson-Norheim E, Hökfelt T (1986) Distribution and origin of substance P- and neuropeptide Y-immunoreactive nerves in the guinea pig heart. *Cell Tissue Res* 243:477-485
18. De Ferrari GM, Vanoli E, Curcuruto P, Tommasini G, Schwartz PJ (1992) Prevention of life threatening arrhythmias by pharmacologic stimulation of the muscarinic receptors by oxo-tremorine. *Am Heart J* 124:883-890
19. De Ferrari GM, Mantica M, Vanoli E, Hull SS, Schwartz PJ (1993) Scopolamine increases vagal tone and vagal reflexes in patients after myocardial infarction. *J Am Coll Cardiol* 22:1327-1334
20. De Ferrari GM, Salvati P, Grossoni M (1993) Pharmacologic modulation of the autonomic nervous system in the prevention of sudden death. A study with propranolol, metacholineand oxotremorine in conscious dogs with a healed myocardial infarction. *J Am Coll Cardiol*22:283-290
21. De Marneffe M, Jacobs P, Haardt R, Englert M (1986) Variations of normal sinus node function in relation to age: role of autonomic influence. *Eur Heart J* 7:662-672
22. De Neef KJ, Jansen JRC, Versprille A (1983) Attenuation of the negative chronotropic effect of myelinated fibers by non-myelinated fibers in the vagus nerve of the rabbit. *Circ Res* 52:508-514
23. Domschke S, Domschke W (1982) Biological actions of vasoactive intestinal peptide in man. In: Said SI (Ed) *Vasoactive Intestinal Peptide.* Raven Press, New York, p. 201-209
24. Donald DE, Samueloff SL, Ferguson D (1967) Mechanisms of tachycardia caused by atropine in conscious dogs. *Am J Physiol* 212:901-910
25. Eckberg DL, Drabinsky M, Braunwald E (1971) Defective cardiac parasympathetic control in

patients with heart disease. *N Engl J Med* 285:877-883
26. Eger EI (1962) Atropine, scopolamine, and related compounds. *Anesthesiology* 23:365-383.
27. Evans JM, Randall DC, Funk JN, Knapp CF (1990) Influence of cardiac innervation on intrinsic heart rate in dogs. *Am J Physiol* 258:H1132-H1137
28. Fahrenkrug J (1993) Transmitter role of vasoactive intestinal peptide. *Pharmacol Toxicol* 72:354-363
29. Forssmann WG, Triepel J, Daffner C, Heym Ch, Cuevas P, Noble MIM, Yanaihara N (1988) Vasoactive intestinal peptide in the heart. *Ann NY Acad Sci* 527:405-420
30. Frase LL, Gaffney FA, Lane LD, Buckey JC, Said SI, Blomqvist CG, Krejs GJ (1987) Cardiovascular effects of vasoactive intestinal peptide in healthy subjects. *Am J Cardiol* 60:1356-1361
31. Goldstein DS (1983) Arterial baroreflex sensitivity, plasma catecholamines, and pressor responsiveness in essential hypertension. *Circulation* 68:234-240
32. Goldstein DS, Kopin IJ (1990) The autonomic nervous system and catecholamines in normal blood pressure control and in hypertension. In: Laragh JH, Brenner BM (Eds) *Hypertension: Pathophysiology, Diagnosis, and Management*. Raven Press, New York, p. 711-747
33. Hall GT, Gardner TD, Potter EK (1990) Attenuation of long lasting effects of sympathetic stimulation after repeated stimulation. *Circ Res* 67:193-198
34. Haney HF, Lindgren AJ (1945) The effect of acetylcholine on the atropinized, denervated heart. *Am J Physiol* 145:177-180
35. Henning RJ (1992) Vagal stimulation during muscarinic and $\beta$-adrenergic blockade increases atrial contractility and heart rate. *J Auton Nerv Syst* 40:121-130
36. Hill MRS, Wallick DW, Martin PJ, Levy MN (1990) Excess tachycardia: an anesthetized preparation and its stimulation frequency response. *Proc Annu Int Conf IEEE Eng Med Biol Soc* 12:757-758
37. Hill MRS, Wallick DW, Martin PJ, Levy MN (1993) Frequency dependence of vasoactive intestinal polypeptide release and vagally induced tachycardia in the canine heart. *J Auton Nerv Syst* 43:117-122
38. Hodes R (1939) Exercise in the sympathectomized cat. *Am J Physiol* 126:171-179
39. Hoffmann F, Hoffmann EJ, Middleton S, Talesnik J (1945) The stimulating effect of acetylcholine on the mammalian heart and the liberation of an epinephrine-like substance by the isolated heart. *Am J Physiol* 144:189-198
40. Hoover DB, Hancock JC (1988) Distribution of substance P binding sites in guinea pig heart and pharmacological effects of substance P. *J Auton Nerv Syst* 23:189-197
41. Jose AD, Collison D (1970) The normal range and determinants of the intrinsic heart rate in man. *Cardiovasc Res* 4:160-167
42. Jose AD, Stitt F, Collison D (1970) The effects of exercise and changes in body temperature on the intrinsic heart rate in man. *Am Heart J* 79:488-498
43. Jourdan F, Nowak SJG (1936) Étude expérimentale chez le chien des fibres cardio-accélératrices du vague. *Arch Int Pharmacodyn Ther* 53:121-135
44. Kabat H (1940) The cardio-accelerator fibers in the vagus nerve of the dog. *Am J Physiol* 128:246-257
45. Karasawa Y Furukawa Y, Ren L, Takei M, Murukami M, Narita M, Chiba S (1990) Cardiac responses to VIP and VIP-ergic-cholinergic interaction in isolated dog heart preparations. *Eur J Pharmacol* 187:9-17

46. Katayama Y, Nishi S (1986) Peptidergic transmission. In: Karczmar AG, Koketsu K, Nishi S (Eds) *Autonomic and enteric ganglia. Transmission and its Pharmacology.* Plenum Press, New York London, p. 181-200
47. Kjekshus JK, Blix AS, GrØttum P, Aasen AO (1981) Beneficial effects of vagal stimulation on the ischaemic myocardium during beta-receptor blockade. *Scand J Clin Lab Invest* 41:383-389
48. Konopka LM, Merriam LA, Hardwick JC, Parsons RL (1992) Aminergic and peptidergic elements and actions in a cardiac parasympathetic ganglion. *Can J Physiol Pharmacol* 70:S32-S43
49. Kumada M, Terui N, Kuwaki T (1990) Arterial baroreceptor reflex: its central and peripheral neural mechanisms. *Prog Neurobiol* 35:331-361
50. Levy MN (1971) Sympathetic-parasympathetic interactions in the heart. *Circ Res* 29:437-445
51. Lovell N, Celler B (1992) Investigation of postvagal tachycardias by analysis of the dynamics of cardiac period responses. *Proc Ann Int Conf IEEE Eng Medic Biol Soc* p. 441-442
52. Lundberg JM, Franco-Cereceda A, Hua X, Hökfelt T, Fisher JA (1985) Co-existence of substance P and calcitonin gene-related peptide-like immunoreactivities in sensory nerves in relation to cardiovascular and bronchoconstrictor effects of capsaicin. *Eur J Pharmacol* 108: 315-319
53. Machado BH, Krieger EM, Salgado HC (1987) Changes of the intrinsic heart rate during the onset of renal hypertension. *J Hypertens* 5:755-759
54. Machado BH, Brody MJ (1989) Contribution of neurogenic mechanisms to control of intrinsic heart rate. *Am J Physiol* 256:R231-R235
55. McWilliam PN, Woolley DC (1990) The effect of supranodose vagotomy on the hexamethonium-resistent bradycardia in the anaesthetized rabbit. *J Auton Nerv Syst* 29:227-230
56. Marcus B, Gilette PC, Garson A Jr (1990) Intrinsic heart rate in children and young adults: an index of sinus node function isolated from autonomic control. *Am Heart J* 119:911-916
57. Moore RM, Cannon WB (1930) The heart rate of unanesthetized normal, vagotomized, and sympathectomized cats as affected by atropine and ergotoxine. *Am J Physiol* 94:201-208
58. Moriarty M, Potter EK, McCloskey DI (1993) Pharmacological separation of cardio-accelerator and vagal inhibitory capacities of sympathetic nerves. *J Auton Nerv Syst* 43:7-16
59. O'Brien IAD, O'Hare P, Corrall RJM (1986) Heart rate variability in healthy subjects: effect of age and the derivation of normal ranges for tests of autonomic function. *Br Heart J* 55:348-354
60. Potter EK (1985) Prolonged non-adrenergic inhibition of cardiac vagal action following sympathetic stimulation: neuromodulation by neuropeptide Y? *Neurosci Lett* 54:117-121
61. Potter EK (1988) Neuropeptide Y as an autonomic neurotransmitter. *Pharmac Ther* 37: 251-273
62. Priola DV, Fulton RL (1969) Positive and negative inotropic responses of the atria and ventricles to vagosympathetic stimulation in the isovolumic canine heart. *Circ Res* 25:265-273
63. Priola DV (1972) Positive dromotropic effects of the vagus nerves on the canine atrio-ventricular node. *Cardiovasc Res* 6:155-162
64. Priola DV, Cao X, Smith DC (1994) Modulation of autonomic responses in normal and denervated isolated canine atria by substance P. *Am J Physiol* 266:H1401-H1407
65. Prystowsky EN, Zipes DP (1985) Postvagal tachycardia. *Am J Cardiol* 55:995-999
66. Randall D, Randall WC, Brown DR, Yingling JD, Raisch R (1992) Heart rate control in awake dog after selective SA-nodal parasympathectomy. *Am J Physiol* 262:H1128-H1135
67. Rechardt L, Aalto-Setälä K, Purjeranta M, Pelto-Huikko M, Kyösola K (1986) Peptidergic innervation of human atrial myocardium: an electron microscopical and immunocyto-chemical study. *J Auton Nerv Syst* 17:21-32

68. Rigel DF, Lipson D, Katona PG (1984) Excess tachycardia: heart rate after antimuscarinic agents in conscious dogs. *Am J Physiol* 246:H168-H173
69. Rigel DF, Katona PG (1986) Effects of antihistamines and local anesthetics on excess tachycardia in conscious dogs. *J Pharmacol Exp Ther* 238:367-371
70. Rigel D F (1988) Effects of neuropeptides on heart rate in dogs: comparison of VIP, PHI, NPY, CGRP and NT. *Am J Physiol* 255:H311-H317
71. Robberecht P, Waelbroeck M, Dehaye JP, Winand J, Vandermeers A, Vandermeers-Piret MC, Christophe J (1984) Evidence that helodermin, a newly extracted peptide from gila monster venom, is a member of the secretin/VIP/PHI family of peptides with an original pattern of biological properties. *FEBS* 166:277-282
72. Roossien A, Brunsting JR, Nijmeijer A, Zaagsma J, Zijlstra WG (1992) Vagal cardioacceleration and sympathetic activity. *Proceedings 7th International Catecholamine Symposium,* Amsterdam p. 275
73. Ross G (1970) Cardiovascular effects of secretin. *Am J Physiol* 218:1166-1170
74. Rutherford W, (1869) Influence of the vagus upon the vascular system. *J Anat Physiol*3:402-416
75. Samaan A (1935) Muscular work in dogs submitted to different conditions of cardiac and splanchnic innervations. The antagonistic cardiac nerves and heart rate. *J Physiol* 83:313-340
76. Schuil HA, Brunsting JR, Van der Molen H, Zijlstra WG (1981) Cardioacceleratory effect of muscarinic blocking agents in the dog. *Eur J Pharmacol* 69:229-233
77. Schuil HA (1987) *Vagale cardio-acceleratie en denervatie van het hondehart.* Thesis, University of Groningen, Van Denderen, Groningen, The Netherlands
78. Skoftisch G, Jacobowitz D (1985) Calcitonin gene-related peptide coexists with substance P in capsaicin sensitive neurons and sensory ganglia of the rat. *Peptides* 6:747-754
79. Smitherman TC, Dehmer GJ, Said SI (1988) Vasoactive intestinal peptide as a coronary vasodilator. *Ann NY Acad Sci* 527:421-429
80. Smitherman TC, Sakio H, Geumei AM, Yoshida T, Oyamada M, Said SI (1982) Coronary vasodilator action of VIP. In: Said SI (Ed) *Vasoactive Intestinal Peptide.* Raven Press, New York, p. 169-176
81. Sneddon JF, Bashir Y, Ward DE (1993) Vagal stimulation after myocardial infarction: accentuating the positive. *J Am Coll Cardiol* 22:1335-1337
82. Stramba-Badiale M, Vanoli E, De Ferrari GM, Cerati D, Foreman RD, Schwartz PJ (1991) Sympathetic-parasympathetic interaction and accentuated antagonism in conscious dogs. *Am J Physiol* 260:H335-H340
83. Vanoli E, De Ferrari GM, Stramba-Badiale M, Hull SS Jr, Foreman RD, Schwartz PJ (1991) Vagal stimulation and prevention of sudden death in conscious dogs with healed myocardial infarction. *Circ Res* 68:1471-1481
84. Vatner SF, Higgins CB, Braunwald E (1974) Sympathetic and parasympathetic components of reflex tachycardia induced by hypotension in conscious dogs with and without heart failure. *Cardiovasc Res* 8:153-161
85. Warner MR, Levy MN (1989) Inhibition of cardiac vagal effects by neurally released and exogen ous neuropeptide Y. *Circ Res* 65:1536-1546
86. Warner MR, Levy MN (1989) Neuropeptide Y as a putative modulator of the vagal effects on heart rate. *Circ Res* 64:882-889
87. Weihe E, Reinecke M, Forssmann WG (1984) Distribution of vasoactive intestinal polypeptide-like immunoreactivity in the mammalian heart. *Cell Tissue Res* 236:527-540

88. Xi X, Duff MJ, Weber M, Fiscus RR, Thomas JX, O'Toole MF, Randall WC (1989) In vivo effects of calcitonin gene-related peptide on intracardiac vagal ganglia. *Faseb J* 3:A413
89. Yamasaki S, Stolfi A, Mas MS, Fox LS, Pickoff AS (1991) Rapid attenuation ("Fade") of the chronotropic response during vagal stimulation in the canine newborn. Evidence for a prominent neuropeptide Y effect. *Circ Res* 69:406-413
90. Yang T, Jacobstein MD, Levy MN (1985) Sustained increases in heart rate induced by time repetition of vagal stimulation in dogs. *Am J Physiol* 249:H703-H709
91. Yang T, Levy MN (1992) Sequence of excitation as a factor in sympathetic-parasympathetic interactions in the heart. *Circ Res* 71:898-905

# Samenvatting

Het zoogdierhart is een autonoom orgaan: de prikkel die aanleiding is tot contractie, ontstaat in het hart zelf in een klein gebied in de wand van het rechter atrium, de sinusknoop. De frequentie van de elkaar opvolgende contracties en relaxaties van de hartspier, die voor een effectieve pompwerking noodzakelijk is, wordt bepaald door de sinusknoop. Om aan de momentane behoefte van het lichaam aan bloed te voldoen wordt de activiteit van de sinusknoop gemoduleerd door het centrale zenuwstelsel. Klassiek worden twee nerveuze invloeden op de hartfrequentie onderscheiden: activering van de parasympathische vezels van de N. vagus veroorzaakt een verlaging van de hartfrequentie, terwijl activering van de (ortho)sympathische vezels van de Nn. accelerantes een verhoging veroorzaakt, respectievelijk aangeduid als het vagale cardio-deceleratoire (VCD) systeem en het sympathische cardio-acceleratoire (SCA) systeem.

Onderzoek in verschillende zoogdieren heeft aannemelijk gemaakt dat de N. vagus naast deceleratoire vezels ook vezels bevat die bij activering een acceleratie van de hartfrequentie bewerkstelligen. Ofschoon dit vagale cardio-acceleratoire (VCA) systeem reeds meer dan een eeuw geleden voor het eerst werd beschreven (Wundt 1859), is zijn bestaan nog steeds niet algemeen bekend en controversieel. Enerzijds doordat de fysiologische relevantie van het VCA systeem niet duidelijk is, anderzijds doordat het VCA systeem niet past in het vigerende concept dat het autonome zenuwstelsel bestaat uit twee anatomisch en fysiologisch gescheiden entiteiten, sympathicus en parasympathicus, die een tegengestelde invloed op de hartfrequentie uitoefenen.

Het onderzoek beschreven in dit proefschrift richtte zich primair op de bestudering van het VCA systeem. De activiteit van het VCA systeem kon onder meer worden aangetoond door farmacologisch de invloed van het SCA-systeem te blokkeren met een $\beta$-adrenerge receptor antagonist en de invloed van het VCD systeem te blokkeren met een muscarinerge receptor antagonist. De activiteit van het VCA systeem kan worden gekwantificeerd als het verschil tussen de frequentie na gecombineerde $\beta$-adrenerge ($\beta$-blokkade) en muscarinerge (M-blokkade) receptor blokkade en de frequentie na additionele vagotomie. In een voorgaand onderzoek is gebleken dat de activiteit van het VCA systeem ook verdwijnt wanneer na gecombineerde $\beta$- en M-blokkade de ganglionaire nicotinerge receptoren worden geblokkeerd (N-blokkade). De activiteit van het VCA systeem kan derhalve ook worden gekwantificeerd als het verschil tussen de frequentie na gecombineerde $\beta$- en M-blokkade en de frequentie na additionele de

N-blokkade.

Uit ons onderzoek bleek dat de VCA activiteit het grootst is enkele minuten na gecombineerde $\beta$- en M-blokkade en vervolgens geleidelijk afneemt. Drie uur na gecombineerde $\beta$- en M-blokkade was de VCA activiteit voor ongeveer 80% verdwenen. Wanneer vervolgens vagotomie wordt verricht en de Nn.vagi elektrisch worden gestimuleerd blijkt er weer een toename in hartfrequentie op te treden. Op grond van de waarneming dat de door stimulatie geïnduceerde frequentietoename kleiner wordt naarmate het tijdsinterval tussen de farmacologische blokkade en de vagotomie langer wordt, wordt verondersteld dat additionele perifere factoren een belangrijke rol spelen in deze geleidelijke afname van de VCA activiteit. Bovengenoemde experimenten zijn uitgevoerd bij honden onder neurolept-anesthesie aangezien deze vorm van anesthesie, in tegenstelling tot pentobarbital-, methoxyflurane- en $\alpha$-chloralose-anesthesie, geen vagolytische neveneffecten heeft.

Eén van de doelstellingen van het onderzoek was het aantonen van de reflectoire activering van het VCA systeem. In een voorgaand onderzoek bij honden onder neurolept-anesthesie was gebleken dat na gecombineerde $\beta$- en M-blokkade een verandering in de arteriële bloeddruk niet tot een eenduidige verandering in de hart-frequentie leidde. Nader onderzoek bracht de neurolept-anesthesie zelf als interfererende factor aan het licht. De reflectoire activering van het VCA systeem werd derhalve verder onderzocht met getrainde wakkere honden bij wie ruim te voren veneuze en arteriële catheters waren geïmplanteerd. Hierdoor konden farmaca intraveneus worden toegediend en de arteriële bloeddruk continu worden geregistreerd. Arteriële bloeddrukstijging werd teweeggebracht met vasopressine of de $\alpha_1$-adrenerge receptor agonist methoxamine, terwijl voor de inductie van een arteriële bloeddrukdaling natrium-nitroprusside of de $\alpha_1$-adrenerge receptor antagonist doxazosin werd gegeven. Ná $\beta$-blokkade, maar vóór de M-blokkade, veroorzaakte een stijging van de arteriële bloeddruk, conform de klassieke baroreceptorreflex, een daling van de hartfrequentie, en vice versa. Na gecombineerde $\beta$- en M-blokkade werd echter een paradoxale reactie gevonden: een stijging van de arteriële bloeddruk resulteerde in een stijging van de hartfrequentie, een daling van de bloeddruk in een daling van de hartfrequentie. De reflectoire (in)activering van het VCA systeem was hiermee aangetoond. Een extra aanwijzing voor deze reflectoire activeerbaarheid was dat een bloeddrukstijging resp. daling die werd geïnduceerd ná $\beta$-blokkade, maar vóór de M-blokkade, resulteerde in een verhoogde resp. verlaagde VCA activiteit ná M-blokkade. Dat de reacties in de hartfrequentie reflectoir van aard zijn en niet rechtstreeks door de bovengenoemde vasoactieve stoffen zelf worden geïnduceerd, blijkt uit de bevinding dat toediening van dezelfde stoffen aan wakkere honden met geblokkeerde $\beta$-adrenerge en muscarinerge receptoren, bij wie een dag van te voren vagotomie was

verricht, niet tot noemenswaardige veranderingen in de hartfrequentie leidde. Omdat zowel bij het lam als de rat eenzelfde relatie tussen arteriële bloeddruk en hartfrequentie als bij de hond werd gevonden, werd geconcludeerd dat ook bij deze diersoorten het VCA systeem aanwezig is. Ofschoon in de literatuur aanwijzingen zijn dat ook bij de mens het VCA systeem bestaat, is dit tot heden nog niet met zekerheid vastgesteld.

Een discrepantie die naar voren kwam bij de vergelijking van de VCA activiteit bij honden in de wakkere en in neurolept-anesthesie, was dat de hartfrequentie na gecombineerde $\beta$-, M- en N-blokkade significant lager is bij de wakkere hond. Het feit dat in genarcotiseerde honden de hartfrequentie na $\beta$-, M-blokkade en vagotomie significant lager is dan die na $\beta$-, M- en N-blokkade en dat deze overeen komt met die na $\beta$-, M- en N-blokkade bij wakkere honden, doet vermoeden dat tijdens neurolept-anesthesie de additionele N-blokkade de VCA activiteit niet volledig elimineert. Een andere aanwijzing hiervoor is dat een bloeddrukstijging geïnduceerd na gecombineerde $\beta$-, M- en N-blokkade zowel bij de hond als bij de rat een toeneming van hartfrequentie geeft. Het gedeeltelijk (narcose) of volledig (wakker) wegvallen van de VCA activiteit na de additionele N-blokkade zou verband kunnen houden met de daling in de arteriële bloeddruk die optreedt door de N-blokkade. Dit zou betekenen dat N-blokkade de VCA activiteit reflectoir vermindert.

Een ander doel van het onderzoek was de karakterisering van de neurotransmitter en de daarbij behorende receptor in het VCA systeem. Hiertoe werden de chronotrope effecten van verschillende farmaca onderzocht door bij wakkere honden na $\beta$- en M-blokkade deze in een lage concentratie vlak bij de SA-knoop intra-arterieel toe te dienen. Veranderingen in hartfrequentie zijn dan uitsluitend een direct effect en kunnen niet worden toegeschreven aan een eventueel reflectoir effect van deze stoffen. Om de lokale toediening van stoffen bij wakkere honden mogelijk te maken werd een canule-catheter systeem ontwikkeld dat permanent in de rechter coronairarterie kan worden geïmplanteerd. Het bleek dat intracoronaire toediening van acetylcholine aan $\beta$- en M-geblokkeerde honden niet tot veranderingen in de hartfrequentie leidde wat erop wijst dat de VCA activiteit niet door een nicotinerge receptor wordt gemedieerd en steunt daarmee onze hypothese dat het wegvallen van de VCA activiteit na N-blokkade reflectoir wordt veroorzaakt door de begeleidende daling in de bloeddruk. De matige toename van de hartfrequentie na intracoronaire toediening van histamine maakt aannemelijk dat histamine niet betrokken is bij het tot stand komen van VCA activiteit. Het bifasische karakter van de frequentiestijging bij hoge doseringen kan worden verklaard door een combinatie van de positieve chronotrope invloed van histamine en de reflectoir geïnduceerde vermindering van de VCA activiteit die het gevolg is van de door histamine veroorzaakte daling in de arteriële bloeddruk. In tegenstelling tot histamine blijkt

*vasoactive intestinal polypeptide* (VIP) reeds bij een zeer lage dosis een sterke stijging van de hartfrequentie te veroorzaken (zonder dat de arteriële bloeddruk noemenswaardig veranderde). Om verschillende redenen wordt VIP als mogelijke neurotransmitter van het VCA systeem gezien. De VIP-immunoreactiviteit in vagale vezels is hoog; VIP is gecolocaliseerd met acetylcholine en blijkt vrij te komen als vagale efferenten worden gestimuleerd met een frequentie van ongeveer 20 Hz. Uit ons onderzoek bleek dat stimulatie van de n. vagus met deze frequentie na M-blokkade tot cardio-acceleratie leidt. Bovendien bleek niet alleen dat VIP een aanzienlijke stijging van de hartfrequentie veroorzaakte, maar tevens dat de grootte van de tachycardie omgekeerd evenredig was met de VCA activiteit op het moment van toediening. Ook bleek dat VIP de hartfrequentie minder laat stijgen wanneer de VCA activiteit reflectoir wordt verhoogd door een (methoxamine-geïnduceerde) bloeddrukstijging. Het sluitende bewijs dat VIP de verantwoordelijke VCA eindtransmitter is, kon nog niet worden geleverd doordat een specifieke cardiale VIP-antagonist nog niet bestaat.

# List of Publications

Bouhuys AN, Beersma D, Hoofdakker vd R, Roossien A (1987) The prediction of short and long term improvement in depressive patients: ethological methods of observing behavior versus clinical rating. *Ethology and Sociology* 8:117-130

Scheurink AJW, Steffens AB, Roossien A, Balkan B (1992) Sympathoadrenal functioning in genetically obese Zucker rats. *Physiology and Behavior* 52:679-685

Haanstra R, Brunsting JR, Roossien A (1988) Excess tachycardia in the dog: influence of the sequence of the drug administration. *Pflügers Arch* 412:S-6

Roossien A, Brunsting JR, Zijlstra WG (1990) Vagal cardioacceleration in conscious and neuroleptanaesthetized dogs. *Pflügers Arch* 416:S-7

Roossien A, Brunsting JR, Nijmeijer A, Zijlstra WG (1991) Responses of the vagal cardioacceleratory system to pharmacologically induced changes in arterial blood pressure. *Pflügers Arch* 419:R-9

Roossien A, Brunsting JR, Zaagsma J, Zijlstra WG (1992) Vasoactive intestinal polypeptide: a probable mediator of vagal cardio-acceleration (VCA) in dogs. *Pflügers Arch* 420:R-88

Roossien A, Brunsting JR, Nijmeijer A, Zaagsma J, Zijlstra WG (1992) Vagal cardioacceleration and sympathetic activity. *Abstracts 7th International Catecholamine Symposium*, Amsterdam

Roossien A, Brunsting JR, Nijmeijer A, Zijlstra WG (1992) The vagal cardioacceleratory system in conscious lambs. *Pflügers Arch* 424:R-41

Nijmeijer A, Brunsting JR, Roossien A (1992) Permanent cannulation of the right coronary artery in the dog. *Abstracts Congress of the International Animal Technology*, Swansea

Roossien A, Brouwer MC, Brunsting JR, Leest J vd, Zijlstra WG (1993) Vagal cardioaccelerator activity in freely moving rats. *Pflügers Arch* 424:R-7

Brunsting JR, Roossien A, Zaagsma J, Zijlstra WG (1993) Non-cholinergic ganglionic transmission of reflexly induced vagal cardioaccelerator impulses in dogs. *Abstracts XXXII Congress of the International Union of Physiological Sciences* 173.21/P, Glasgow

Roossien A, Brunsting JR, Zaagsma J, Zijlstra WG (1993) Further evidence for non-cholinergic ganglionic transmission in the vagal cardioaccelerator pathway in dogs. *Abstracts XXXII Congress of the International Union of Physiological Sciences* 173.23/P, Glasgow

Nijmeijer A, Brunsting JR, Roossien A (1993) Permanent cannulation of the right coronary artery in the dog. *Abstracts Congress of the International Animal Technology*, Lancaster

Roossien A, Brunsting JR, Nijmeijer A, Schuil HA, Zijlstra WG. Mechanism of the decline in vagal cardiac acceleration in dogs in neuroleptanesthesia. *J Auton Nerv Syst* (accepted for publication)

Nijmeijer A, Roossien A, Brunsting JR (1994) Permanent cannulation of the right coronary artery in the dog. *Animal Technology* 45:(in press)

Roossien A, Brunsting JR, Nijmeijer A, Zaagsma J, Zijlstra WG. Reflex control of the vagal cardiac accelerator system in conscious dogs. *J Auton Nerv Syst* (submitted)

Roossien A, Brunsting JR, Nijmeijer A, Zaagsma J, Zijlstra, WG. Vasoactive intestinal polypeptide; a probable mediator in the vagal cardiac accelerator system in dogs. *Circulation* (submitted)

Roossien A, Brouwer MC, Brunsting JR, Zaagsma J, Zijlstra WG. Reflex activation of the vagal cardiac accelerator system in conscious dogs, lambs and rats. (in preparation)

Roossien A, Brunsting JR, Nijmeijer A, Zaagsma J, Zijlstra WG. Influence of neuroleptanesthesia on the vagal cardiac acceleration. (in preparation)

# Dankwoord

Dit proefschrift is een produkt van vele jaren werk, werk dat nutuurlijk is uitgevoerd samen met anderen. Ter afsluiting wil ik dan ook graag iedereen bedanken die op enigerlei wijze heeft bijgedragen aan het tot stand komen van dit eindprodukt, in het bijzonder de co-promotor Jos Brunsting voor de prettige en leerzame samenwerking; daarnaast dank Prof. W.G. Zijlstra voor alle verhelderende discussies en voor het snel en kritisch doornemen van de manuscripten; Prof J. Zaagsma voor het nakijken van de manuscripten en het plaatsen van kritische noten. Arie Nijmeijer dank ik voor al het biotechnisch werk en voor de gezellige uren die we samen tijdens de proeven (veelal in het donker) doorbrachten. Jaap Muntinga ben ik zeer erkentelijk voor zijn hulp op het gebied van de software en voor de vele stimulerende discussies. Verder dank ik Henk v.d. Molen en Piet Schiphof voor de biotechnische ondersteuning en de verzorging van de dieren; Wilma Meeuwsen-van de Roest voor haar analytisch werk; Mart Lubben-Wehrmeijer en Lenny Anthonio voor het uitwerken van de proefverslagen (die steeds langer en langer werden) en voor al het secretariële werk; Jan Schut voor zijn ondersteuning op het gebied van de elektronica en Greet Popken voor haar inspanningen om de veelal oude literatuur te achterhalen. Henk Schuil en Bert Benthem ben ik zeer erkentelijk voor de plezierige samenwerking en voor hun (ongevraagde) aan- en opmerkingen; Wieb Patberg en Jaap v.d. Leest voor hun steun bij het tot stand komen van de figuren; Rob Coppes voor de catecholamine bepalingen en Sybolt de Vries voor zijn assistentie bij het analyseren van de uitkomsten ervan. Van de vakgroep dierfysiologie wil ik speciaal Christien Brouwer bedanken voor de prettige samenwerking bij het uitvoeren van de experimenten met ratten en de overige leden van vleugel D voor alle gezelligheid, gastvrijheid en voor het verbreden van mijn wetenschappelijke kennis en interesse. De leden van de beoordelingscommissie, Prof. Dr. B. Bohus, Prof. Dr. J.R.G. Kuipers en Prof. Dr. K.I. Lie, dank ik voor het vlot lezen en beoordelen van het manuscript. Tot slot wil ik mijn familie, vrienden en kennissen bedanken voor hun interesse en hun steun met name tijdens de schrijfdagen. Heel in het bijzonder dank ik Pé voor al het vertrouwen en voor Nick.